Use of Humidified Air in Optimizing APCI−MS Response in Breath Analysis

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An atmospheric-pressure chemical ionization−mass spectrometer (APCI−MS) system was modified to introduce gaseous samples as well as to humidify sheath and auxiliary gases. Solutions of single odorants in pentane were evaporated inside a vessel in a dry environment (relative humidity 0−10%) and in a human-breath-like environment (relative humidity 88−98%) and subsequently analyzed in the positive ion mode. The results indicated that using dry nitrogen as sheath and auxiliary gases led to strong fragmentation of the molecules combined with a low sensitivity of detection. Humidification of both gases not only increased sensitivity but also resulted in the protonated molecular ion as the base peak in 5 of the 6 compounds studied (ethyl butyrate, 3-methyl-3-phenyl glycidic acid ethyl ester, γ-decalactone, (*E*)-2-hexenal, and cinnamic aldehyde). Only hexanal, which formed a cluster (*m*/*z* 183), did not have the protonated molecular ion as base peak. It was found that the humidity of the sample itself did not have any influence on sensitivity or fragmentation.

Keywords: *APCI−MS; humidification; flavor; aroma release*

INTRODUCTION

The value of mass spectrometry (MS) in medical, forensic, and environmental analysis has long been recognized and has recently expanded to include the analysis of volatile compounds in human breath (Linforth and Taylor, 1993). Breath analysis by mass spectrometry is problematic because of high background competition produced by the moisture, air, carbon dioxide, and metabolites (e.g., acetone, ethanol, and methanol) in the breath. Also, concentrations of aroma compounds in the breath are very low, thereby challenging instrumental limits. Thus, early techniques to measure volatiles in the nose or mouth during eating have involved the use of Tenax trapping (gas chromatography) or membranes (mass spectrometry) to separate aroma compounds from air and moisture. These approaches (e.g., Delahunty et al., 1994; Ingham et al., 1995; Linforth et al., 1996; Roozen and Legger-Huysman, 1995) have demonstrated that the concentrations of flavor compounds in breath change with the length of time the food is in the mouth. Aroma release from a food in the mouth is dependent upon many factors, including the aroma compound being measured, the mastication process, and the food matrix (Overbosch et al., 1991). The measurement of aroma compound release in the mouth as a function of eating time has been termed the time-release profile. Gathering time-release data by Tenax trapping methods is slow and one typically does not get individual breath-by-breath data but pooled data over several breaths. Results suggest that the time release profile is very dynamic and one needs to collect breath-by-breath data to get an accurate picture of this phenomenon. Thus, researchers have sought more rapid measurement techniques.

Passing breath (or food headspace) through traditional MS membrane separators allows the introduction of volatiles into electron impact sources while excluding air and water (Soeting and Heidema, 1988: Springett et al., 1999). These methods enable the analysis of time-release profiles in almost real time, but suffer from selective permeability of the membrane for different compounds. This can complicate both quantification and time profiling.

Alternative methods have been developed which do not utilize membranes to separate the gas sample from the ionization region of the MS. One such method, atmospheric pressure chemical ionization−mass spectrometry (APCI−MS), was first described by Lovett et al. (1979). Unfortunately, the direct introduction of breath into the source caused difficulties due to interferences from ammonia. An improved APCI system for direct sampling of exhaled human breath was described by Benoit (1983). In accordance with Good et al. (1970), Benoit found the generation of protonated water clusters of varying size, which are formed from the moisture in the ambient air. These protonated water clusters result from a series of ion−molecule reactions which are initiated by the ionization of molecular nitrogen in the source. In the second step, a proton-transfer reaction occurs between the analyte and the protonated water cluster, hence forming protonated analyte-water clusters of varying sizes. Finally, the loosely held water is stripped off by induced collisions with neutral nitrogen molecules and the protonated analyte is transmitted to the analyzer. One can see the dependence of the system on high nitrogen flows and water in the source from this brief description of the process.

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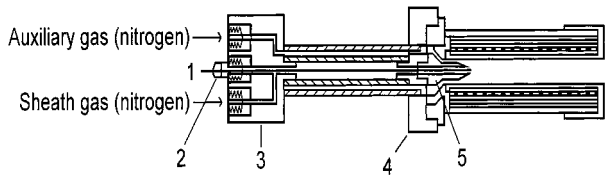

Figure 1. Cross-sectional view of the APCI probe. 1, sample tube; 2, sample inlet fitting; 3, APCI manifold; 4, vaporizing flange; 5, APCI nozzle.

Although APCI−MS has recently been widely used to analyze odorants in real time in human breath (Linforth et al., 1999; Linforth and Taylor, 1998; Davidson et al., 1999), as well as for flavor-release measurements from foods in model systems (Brauss et al., 1999), little detail has been published on the operating conditions used in data collection for these applications. The importance of water as a chemical ionization reagent in APCI−MS analysis was briefly discussed by Sunner et al., (1988b) who reported that increasing the relative humidity of the APCI−MS ionization gases decreased instrument sensitivity. However, our work appears to contradict the findings of Sunner et al., (1988b) in that increased humidity in the ionization gases decreased fragmentation and increased sensitivity. Thus, the purpose of our paper is to elaborate on our observations in this respect. This work is part of a study funded by Robertet Flavors, Inc. (Piscataway, NJ) to understand the role of food ingredients in influencing aroma release from food.

MATERIALS AND METHODS

Chemicals. All compounds used in this study were obtained from Sigma-Aldrich (St. Louis, MO).

APCI Probe-Assembly Modification. To introduce gaseous samples into the ionization chamber in a sufficient quantity, the original APCI probe assembly (Finnigan MAT/ThermoQuest, San Jose, CA) was modified as described below.

The original sample tube (no. 1, Figure 1; fused silica capillary, 0.150 mm i.d., 0.390 mm o.d.) was replaced with a 0.53 mm i.d., 0.73 mm o.d. (total length 30 cm, methyl deactivated) capillary tubing. To allow this modification, a hole (0.78 mm in diameter) was drilled through the sample tube inlet fitting (no. 2), the APCI manifold (no. 3), the vaporizing flange (no. 4), and the APCI nozzle (no. 5). The new larger diameter tube was installed in the instrument as instructed in the instrument manual.

Development of a Vessel for Evaporation of Compounds in Human-Breath-Like Atmosphere. The vessel shown in Figure 2 was made by a glassblower according to our specifications: total volume, 1L; completely water-jacketed inner surface to allow equal heating and to avoid condensation; and top of the vessel with 5 standard sockets (1 × 45/50, 2 × 24/40, 2 × 14/20).

Before use, the glass parts were defatted with acetone and then dried for 2 h at 140 °C. To deactivate the glass surface, the vessel was filled with a 5% (w/w) solution of dimethyldichlorosilane in anhydrous toluene. After 24 h at room temperature, the solution was removed and the vessel was rinsed with toluene, subsequently with methanol, and then with diethyl ether, and dried in a stream of nitrogen.

Determination of the Relative Humidity of Human Breath. To determine the relative humidity of human breath, 3 assessors (2 men, 1 woman) were asked to breath through an attached mask into the glass vessel (Figure 2). To avoid any condensation, the vessel was kept at 38.5 °C. A hygrometer (HANNA Instruments, Rochi di Villafranca, Italy) was inserted into the vessel and the humidity of the breath exhaled through the mouth or nose was monitored continuously until a constant value was obtained (Table 1).

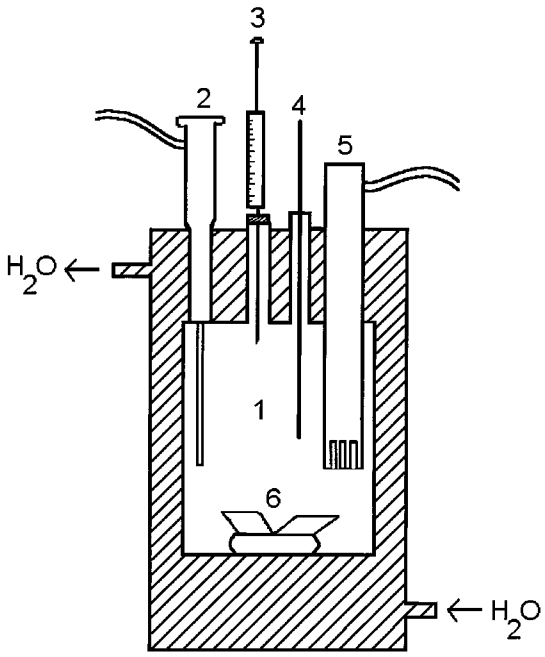

Figure 2. Diagram of the setup used for measurement of evaporated odorants in a human-breath-like atmosphere. 1, water-jacketed glass vessel; 2, thermo-sensor; 3, syringe; 4, capillary tubing; 5, hygrometer; 6, magnetic stirrer bar with 2 blades attached.

Table 1. Measured Relative Humidity of Human Breath

	relative humidity[a](%)	
assessor	nose	mouth
1	85	96
2	82	91
3	85	94

[a] Relative humidity of human breath exhaled through nose and mouth, respectively.

Evaporation of Odorants (Dry). An aliquot (2.5 or 50 μL) of the solutions of odorants was injected through a septum into the glass vessel (no. 1, Figure 2), which was heated to 38.5 °C. To accelerate equilibration of the odorants, the air inside the vessel was continuously circulated by a rotating Teflon-coated magnetic stirrer bar with 2 blades attached (no. 6).

The odorants (presented later in Figures 4-9) were dissolved in pentane (ca. 2 mg/mL). Subsequently, an aliquot (50 μL) of the odorant was injected into the vessel as described above. The concentration of the odorant in the air was calculated assuming 100% evaporated after 3 min and no losses occurred via condensation on the stirrer bar or glass wall.

Evaporation of Odorants (Humidified). To achieve a humidity similar to human breath, water (50 μL) was injected into the vessel (no. 1, Figure 2), which was held at 38.5 °C. To ensure equilibration, the air within the vessel was continuously circulated using a magnetic stirrer bar with 2 blades attached (no. 6) while the temperature and relative humidity were monitored using a thermocouple (no. 2) and a hygrometer (no. 5). After 5 min, an aliquot (2.5 or 50 μL) of the solutions of odorants (Table 1) was evaporated inside the vessel as described above.

APCI Analysis of Evaporated Odorants. Exactly 3 min after adding the test odorant to the sample vessel, the capillary (20 cm × 0.53 mm i.d., Chrompack Netherlands; no. 4, Figure 2) was connected to the sample tube (40 cm, 0.53 mm i.d., Chrompack, Netherlands; no. 1, Figure 2) via a press-fit capillary connector (Hewlett-Packard, Little Falls, PA). To avoid condensation and retention within the sample tube, it was heated to 70 °C. After each run, the vessel was cleaned

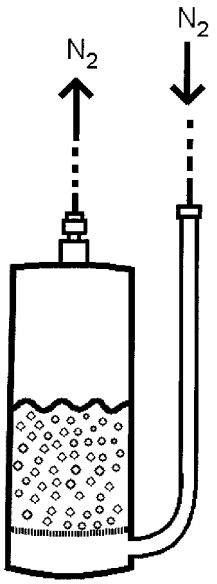

Figure 3. Gas-washing bottle for humidification of sheath and auxiliary gas.

by rinsing it with methanol (3 × 100 mL) and dried by flushing it with hot air (120 °C) for 5 min and clean ambient air for 3 min.

APCI–MS Operating Conditions. The sheath gas (nitrogen, pressure 80 arbitrary units; 5.7L/min) and auxiliary gas (nitrogen, pressure 0 or 60 au; 0 or 7.5L/min) were set as noted. Under these conditions, gas was drawn into the source through the enlarged inlet tube at a flow rate of about 85 mL/min. Mass spectra were obtained in the positive ion mode (corona discharge needle voltage 5 kV, plasma current 5 μA, capillary inlet 15 V) using an ion trap MS (LC-Q, Finnigan MAT/ThermoQuest, San Jose, CA). To increase the relative humidity of the auxiliary and sheath gases, 2 gas-washing bottles (6.5 cm i.d. × 15.5 cm, Figure 3) were made from stainless steel. Each of the bottles was filled with water (80 mL), and the bottles were kept at a constant temperature of 38.5 °C. Humidification was achieved by passing the gas through a frit (pore size 5 μm), resulting in a relative humidity of both gases of ca. 65% after leaving the bottles.

RESULTS AND DISCUSSION

Solvent Choice and Sample Volume. To facilitate the evaporation of known amounts of odorants into air for APCI–MS analysis, we needed to choose a volatile solvent that did not interfere with the analysis of the odorants. Because of its low boiling point and inertness as a saturated hydrocarbon, we decided to use pentane which has also been successfully used for the evaporation of labeled odorants as internal standards (Zehentbauer and Grosch, 1997).

Originally, we chose to use 2.5-μL aliquots of standard solutions for evaporation but found higher standard deviations than desired. Thus, in later data collection we changed to 50-μL aliquots (which were also easier to accurately measure). Neither the overall intensity nor the fragmentation pattern of any compound was significantly affected by the presence of this hydrocarbon. In most cases (ethyl butyrate, ethyl valerate, and *cis*-3-hexenol), a slightly higher overall MS intensity was found when using a 50-μL vs a 2.5-μL injection volume (same amount of test compound). However, these differences were only 3.1% for ethyl butyrate and 2.5% for ethyl valerate. The highest increase of 14.4% was found for *cis*-3-hexenol but these variations were within the standard deviation found for the 2.5-μL injections. On the other hand, the abundances of maltol and isoamyl isovalerate were decreased by 16.1 and 14.3%, respectively, using the larger injection volume. MS response for γ-decalactone stayed the same. As different stock solutions were used in comparison of sample volume, some part of the observed changes may have been due to variation in sample preparation.

As expected, the standard deviation for all compounds decreased by injecting the larger sample volume. The biggest difference was found for *cis*-3-hexenol and ethyl valerate, for which the standard deviation decreased to approximately one-third of its initial value. The standard deviation of all the other compounds was about one-half of that observed for the 2.5-μL injection, except γ-decalactone which showed the same variation for both injection volumes.

On the basis of these results, pentane is considered to be a suitable solvent for the dilution and subsequent evaporation of odorants. Including as much as 50 μL of pentane in the 1-L sample vessel did not significantly alter the MS fragmentation pattern or ion intensity.

Humidification of MS Sheath and Auxiliary Gases – Effect on Esters. Our interest was to

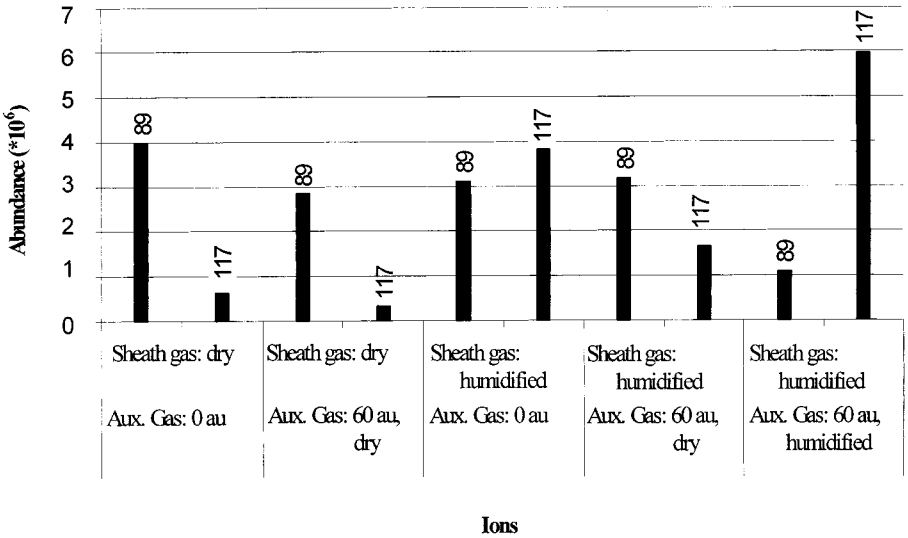

Figure 4. Influence of humidification of sheath (pressure 80 au) and auxiliary gases (pressure 0 or 60 au) on the fragmentation of ethyl butyrate (101.1 μg/L air).

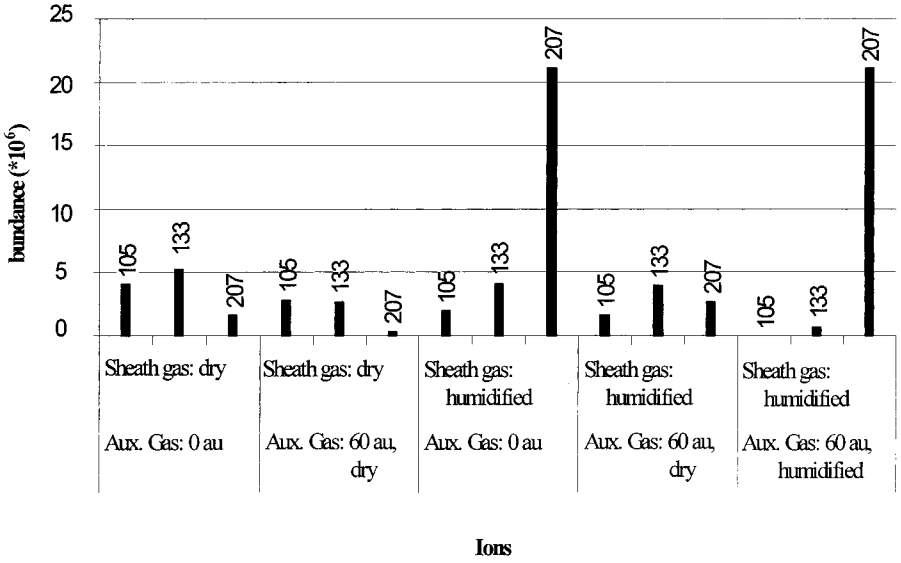

Figure 5. Influence of humidification of sheath (pressure 80 au) and auxiliary gases (pressure 0 or 60 au) on the fragmentation of 3-methyl-3-phenyl glycidic acid ethyl ester (101.75 μg/L air).

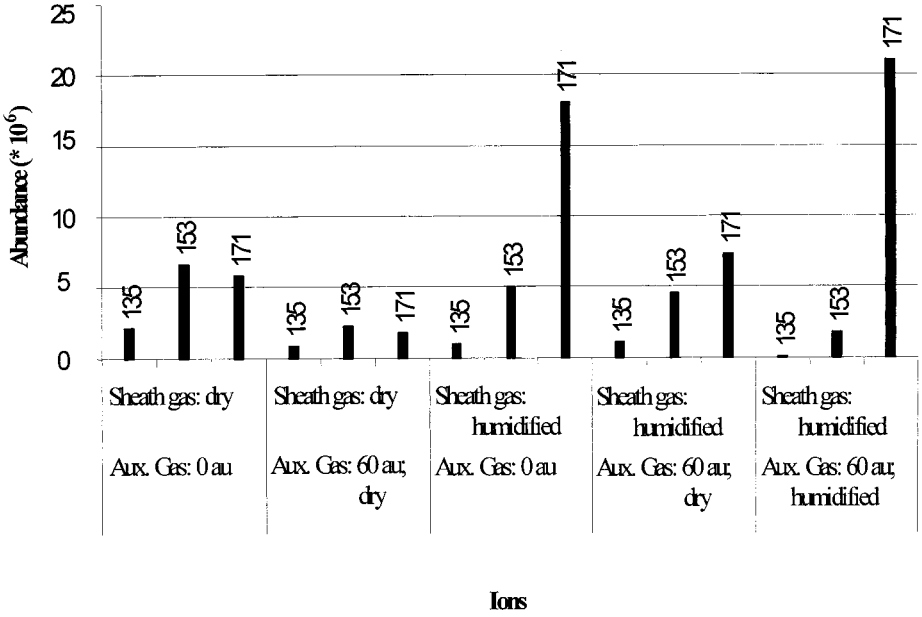

Figure 6. Influence of humidification of sheath (pressure 80 au) and auxiliary gases (pressure 0 or 60 au) on the fragmentation of γ-decalactone (102.5 μg/L air).

investigate the effect of humidification and flow rates of sheath and auxiliary gases on MS sensitivity and fragmentation patterns of selected aroma compounds. This study was performed with three esters (ethyl butyrate, 3-methyl-3-phenyl-glycidic acid ethyl ester, and γ-decalactone) and three aldehydes (hexanal, (*E*)-2-hexenal, and trans-cinnamic aldehyde).

As shown in Figures 4−6 , all three esters followed similar patterns. Under dry conditions, generally a strong fragmentation was observed. Ethyl butyrate was mainly cleaved into butyric acid ($(M+H)^+ = 89$), and the glycidate showed an ion with $m/z = 133$, which was most likely formed by a cleavage between the epoxide and the carbonyl group of the acid. γ-Decalactone, an intramolecular ester, tended to lose water under these conditions.

The additional introduction of dry nitrogen in the form of auxiliary gas (60 au or 7.5 L/min) led in all cases to a decrease in MS intensity, while the specific fragmentation of the compounds stayed the same. It can be assumed that the dilution effect of the auxiliary gas led to this decrease in intensity, suggesting that even at this high concentration of analytes (100 ug/L air) the source was not saturated.

The introduction of additional water in the form of humidified sheath gas led to a drastic change in the fragmentation pattern. In all cases the protonated molecule $(M+H)^+$ ion was the base peak. The biggest effect was found for glycidate for which the $(M+H)^+$ ion ($m/z = 207$) was 77.5% of the sum of all ions formed. For γ-decalactone the $(M+H)^+$ peak was 75%, and for ethyl butyrate it was 55.2%.

The addition of dry auxiliary gas again increased fragmentation. It is assumed that the dilution of humidity caused by the additional dry auxiliary gas is responsible for this change. Nevertheless, compared to the dry experiments an increased $(M+H)^+$ peak could be seen, which is obviously due to the increased water content

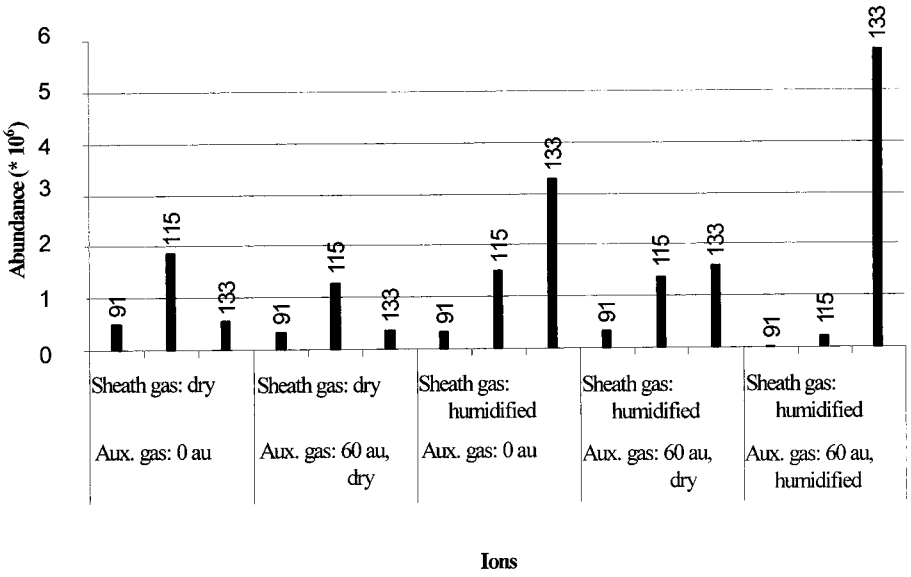

Figure 7. Influence of humidification of sheath (pressure 80 au) and auxiliary gases (pressure 0 or 60 au) on the fragmentation of cinnamic aldehyde (102.1 μg/L air).

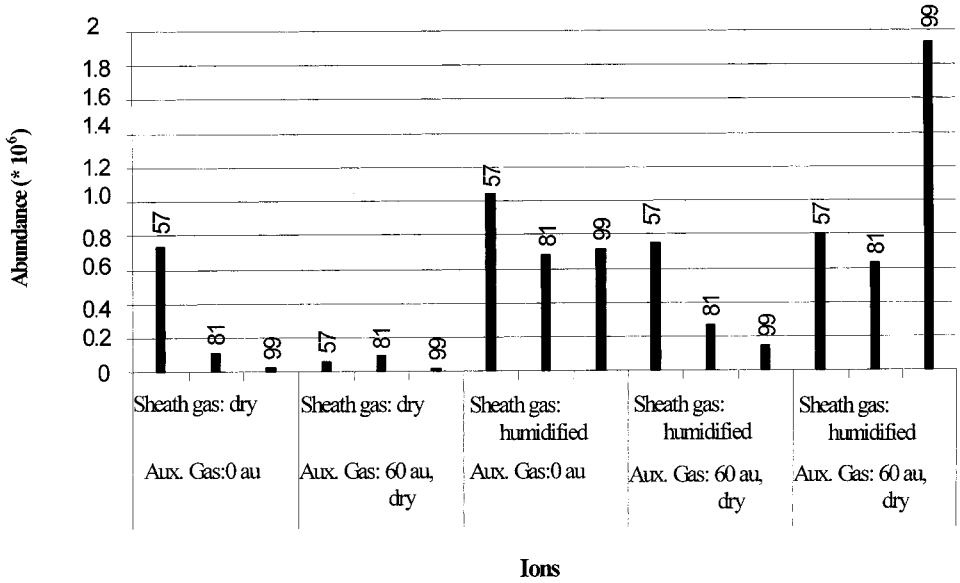

Figure 8. Influence of humidification of sheath (pressure 80 au) and auxiliary gases (pressure 0 or 60 au) on the fragmentation of (*E*)-2-hexenal (100.8 μg/L air).

within the source. Further, because of the dilution a decreased overall intensity (sum of intensities of all ions) was observed.

In the last case, in which both gases (sheath and auxiliary) were humidified, the lowest fragmentation was observed. For ethyl butyrate and γ-decalactone, the highest intensity of the $(M+H)^+$ ion was found for all five gas combinations. The butyric acid fragment in ethyl butyrate was reduced to 16.5% of the total fragmentation and the water cleavage fragment of γ-decalactone amounted to only 8.6%. In the case of glycidate, the intensity of the $(M+H)^+$ peak was the same as when using only humidified sheath gas with no auxiliary gas, however, the overall fragmentation was lower.

Humidification of MS Sheath and Auxiliary Gases − Effect on Aldehydes. The results obtained for the three aldehydes followed trends similar to those discussed above.

Under dry conditions, the aldehydes showed similar fragmentation and generally tended to lose water (Figures 7−9). However, in the case of hexanal two additional ions ($m/z = 117$ and $m/z = 183$) were observed (Figure 9). Because both ions show a higher mass than the molecular ion and sample purity was confirmed by GC (data not shown), they must have been formed by rearrangement or clustering of previously formed fragments. For example, the ion $m/z = 183$ can be explained by a cluster consisting of hexanal (molecular mass = 100) and its protonated water cleavage fragment (m/z = 83).

Humidification of sheath and auxiliary gases was suitable to maximize the intensity of the $(M+H)^+$ ions of the unsaturated aldehydes. However, hexanal was an exception. In the latter case, humidification of sheath gas alone resulted in a 23-fold increase in the m/z = 183 signal, but with both gases humidified, its intensity was decreased. It is suggested that the increased flow rate through the source inhibited the formation of these clusters.

Contribution of Humidity in the Breath to the Ionization Process. The strong effect of the presence

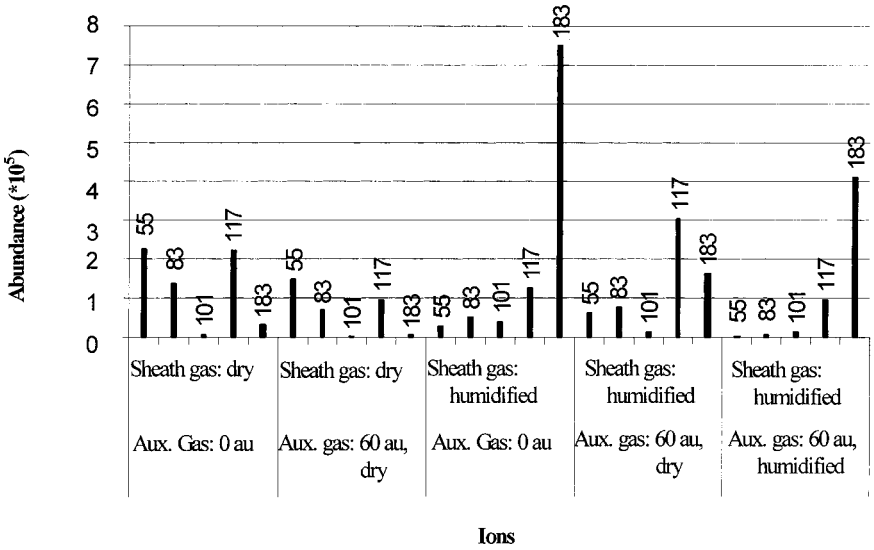

Figure 9. Influence of humidification of sheath (pressure 80 au) and auxiliary gases (pressure 0 or 60 au) on the fragmentation of hexanal (100.5 μg/L air).

Table 2. Fragmentation and Total Intensity of Odorants during APCI Analysis[a] as Influenced by Sample Humidity

		sheath gas dry				sheath gas humidified			
		vessel dry[b]		vessel humidified[c]		vessel dry[b]		vessel humidified[c]	
odorant	conc[d].	int[e]	ions[f]	int[e]	ions[f]	int[e]	ions[f]	int[e]	Ions[f]
ethyl butyrate	100.4	1.86E+6	**89**: 93.7	1.95E+6	**89**: 93.6	3.26E+6	**89**: 49.7	3.86E+6	**89**: 47.9
			117: 6.3		**117**: 6.4		**117**: 50.3		**117**: 52.1
ethyl valerate	103.9	4.25E+6	**103**: 87.1	4.20E+6	**103**: 87.6	1.07E+7	**103**: 57.7	1.16E+7	**103**: 57.0
			131: 12.9		**131**: 12.4		**131**: 42.3		**131**: 43.0
isoamyl isovalerate	101.0	1.36E+6	**103**: 95.2	1.40E+6	**103**: 94.9	3.02E+6	**103**: 46.4	3.18E+6	**103**: 47.8
			173: 4.7		**173**: 5.1		**131**: 53.6		**173**: 52.2
cis-3-hexenol	101.5	4.17E+5	**55**: 49.8	4.19E+5	**55**: 51.6	1.81E+6	**55**: 25.0	1.64E+6	**55**: 23.2
			83: 45.3		**83**: 43.7		**83**: 46.3		**83**: 44.6
			101: 4.9		**101**: 4.7		**101**: 25.0		**101**: 23.2
γ-decalactone	100.9	4.43E+6	**135**: 18.1	5.09E+6	**135**: 17.8	1.11E+7	**135**: 5.3	1.02E+7	**135**: 5.0
			153: 47.8		**153**: 47.1		**153**: 28.4		**153**: 29.5
			171: 34.1		**171**: 35.1		**171**: 66.3		**171**: 65.6

[a] Sheath gas pressure, 80 au; auxiliary gas pressure, 0 au. [b] Relative humidity inside vessel, 0−8%. [c] Relative humidity inside vessel, 90−98%. [d] Concentration (μg/L air) of odorants inside vessel assuming 100% evaporation. [e] Intensity calculated as sum of all major ions. [f] Major ions represented as share (%) of total intensity.

of water during APCI analysis raised the question as to whether moisture in the breath might influence intensity and fragmentation during nose or mouth space analysis compared to the analysis of a dry food product. As shown in Table 1, the relative humidity of human breath exhaled through the nose was 84%. The humidity of breath exhaled through the mouth was about 10% higher, or about 94%.

To evaluate the potential contribution of breath moisture as an APCI gas, single odorants were evaporated in dry and humidified atmospheres and subsequently analyzed using dry and humidified sheath gas, respectively. The results are given in Table 2. The total intensity and the percentage of the ions formed for each odorant were the same for the dry and the humidified vessels. Obviously, the high flow rate of sheath gas resulted in a sufficient dilution of the humidified sample such that no significant difference was noted. However, comparing the results of dry sheath gas with the humidified gas, the same tendencies as discussed above were found. Because of humidification of the sheath gas, a strong increase in the $(M+H)^+$ ion combined with a decrease in the percentage of the fragments was visible.

Discussion. Introducing water into the APCI source in the form of humidified sheath and auxiliary gases improved the sensitivity and decreased fragmentation of our test volatiles. As was noted in the Introduction, this is contrary to the effect reported by Sunner et al., (1988b). We should note that we are operating at much higher analyte concentrations than Sunner et al., (1988) and our ionization source is of different design. Sunner et al., (1988a, b) were working in the low ppb range, and thus, we are working at somewhat higher concentrations than they were. It is conceivable that under higher analyte concentrations, we needed to add more reagent gas (water vapor) to provide ionization of our analytes. Some of the effects we noted may also be the result of different source design. In our system, the sheath and auxiliary gases were mixed with the sample *before* ionization (before the corona discharge needle). The very high flows of dry gases (sheath and auxiliary gases) create an extremely dry ionization environment. There may have been a lack of reagent (water) ions in this environment. In Sunner et al.'s. (1998a,b) instrument, they did not add large gas volumes to their ionization source and their sample stream had 21% relative humidity (at ambient). Thus, they may well have had an adequate amount of reagent gas, and additional water would have had a negative effect. Thus, a different APCI−MS operating at lower analyte con-

centrations and of different design may not benefit as much (or at all) from adding humidity to the ion source.

CONCLUSIONS

We found that introducing water into the APCI source in the form of humidified sheath and auxiliary gases improved the sensitivity and decreased fragmentation of our test volatiles. Whereas under dry conditions the ion intensities were generally low and fragmentation was high, the intensities of the $(M+H)^+$ peaks (with the exception of hexanal) could be maximized by humidification of both gases. Further, due to the large dilution in the ion source, humidity of the sample itself did not have any effect on fragmentation or intensity during APCI analysis. Hence, depending upon the instrument, humidification of the sheath and auxiliary gases may be beneficial when using APCI−MS methods in flavor release measurements.

Hexanal, as a saturated aldehyde, formed clusters with maximum sensitivity using humidified sheath gas only. Further experiments are planned to investigate these clusters thoroughly using MS−MS techniques. Further it appears that a general prediction of the ions observed during analysis may be difficult. Therefore, preliminary experiments are suggested in order to elucidate the ion formation of each compound combined with optimization of the MS operating parameters to maximize intensity and minimize fragmentation. Whereas this paper has considered only the use of humidified gases in the ion source, optimization of other MS operating parameters and reagent gases should be considered to obtain minimal fragmentation and maximum sensitivity.

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