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Direct Analysis of Fatty Acid Vapors in Breath by Electrospray Ionization and Atmospheric Pressure Ionization-Mass Spectrometry

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Real time analysis of human breath is achieved in an atmospheric pressure ionization mass spectrometer (API-MS) by negatively charging exhaled vapors via contact with an electrospray cloud. The spectrum observed is dominated by a wide range of deprotonated fatty acids, including saturated chains up to C14. Above C14, the background from cutaneous sources becomes dominant. We also tentatively identify a series of unsaturated fatty acids (C7−C10), ketomonocarboxylic acids (C6−C10), and a family of aldehydes. The ionization probability of large fatty acids increases drastically when the humidity changes from 20% to 95%. Accordingly, distinguishing lung vapors (humid) from those in the background (dry) requires special precautions. Estimated fatty acid vapor concentrations in breath based on our measurements (∼100 ppt) are in fair agreement with values expected from blood concentrations in the range for which data are available (C3−C6).

The use of breath analysis for medical diagnostic applications was pioneered by Pauling in the early 70s via gas chromatography (GC).[1] Currently, gas chromatography/mass spectrometry (GC/MS) is the standard technique for determining the composition of volatile organic compounds (VOCs) in breath.[2] One drawback of this approach complicating its introduction into clinical practice is that it requires time-consuming sample preparation. Another limitation has been the inability of GC/MS to detect species of molecular weight exceeding 200 Da, many of which would tend to be more biologically relevant than the lighter and more volatile compounds commonly detected. Atmospheric pressure ionization-mass spectrometry (API-MS) has successfully overcome some of these restrictions for the special case of polar species, providing online breath analysis.[3] More recently, different alternatives such as proton transfer reaction-mass spectrometry (PTR-MS) and selected ion flow tube mass spectrometry (SIFT-MS) have also led to online breath studies showing their potential use as diagnostic tools.[4,5]

We have recently demonstrated the high sensitivity to ambient vapors that can be achieved by combining electrospray charging[6−8] with an atmospheric pressure ionization mass spectrometer (API-MS).[9,10] The potential of this approach was first pointed out by Fenn and colleagues,[6,7] at a time when API-MS was considerably less sensitive than it is today. However, the promise of these early observations for breath analysis was not pursued until recently. Our first study on the subject was based on breath vapor protonation by contact with the charged drops produced by electrospraying an acidic solution. It identified urea and a number of relatively heavy species never seen before in the vapor form from breath, as well as in urine.[11] Urea, however, had been identified previously in breath, though interpreted as being transported in aerosol form.[12] More recently we have studied the sensitivity of API-MS to the vapors of the explosives 2,4,6-trinitrotoluene (TNT) and pentaerythritol tetranitrate (PETN) and demonstrated outstanding sensitivities of 0.3 ppt in negative ionization mode.[10] In the present study, we use API-MS to reexamine breath under the negative ionization mode.

EXPERIMENTAL SECTION

The approach is similar to that described previously for breath analysis in the positive mode.[9] The entrance of a quadrupole time of flight mass spectrometer (MDS Sciex QStar) was modified to

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(1) Pauling, L.; Robinson, A. B.; Teranishi, R.; Cary, P. *Proc. Natl. Acad. Sci. U.S.A.* **1971**, *68*, 2374–2376.

(2) Mukhopadhyay, R. *Anal. Chem.* **2004**, *76*, 273A–276A.

(3) Benoit, F. M.; Davidson, W. R.; Lovett, A. M.; Nacson, S.; Ngo, A. *Anal. Chem.* **1983**, *55* (4), 805–807.

(4) Wehinger, A.; Schmid, A.; Mechtcheriakov, S.; Ledochowski, M.; Grabmer, C.; Gastl, G. A.; Amann, A. *Int. J. Mass Spectrom.* **2007**, *265* (1), 49–59.

(5) Smith, D.; Španěl, P. *Mass Spectrom. Rev.* **2005**, *24* (5), 661–700.

(6) Whitehouse, C. M.; Levin, F.; Meng, C. K.; Fenn, J. B. *Proceedings of the 34th ASMS Conference on Mass Spectrometry and Allied Topics*, Cincinnati, OH, June 8−13, 1986; p 507.

(7) Fuerstenau, S.; Kiselev, P.; Fenn, J. B. *Proceedings of the 47th ASMS Conference on Mass Spectrometry and Allied Topics*, Dallas, TX, June 13−17, 1999; ThOE 3:00.

(8) Wu, C.; Siems, W. F.; Hill, H., Jr. *Anal. Chem.* **2000**, *72*, 396–403.

(9) Martínez-Lozano, P.; Fernández de la Mora, J. *Int. J. Mass Spectrom.* **2007**, *265*, 68–72.

(10) Martínez-Lozano, P.; Rus, J.; Fernández de la Mora, G.; Hernández, M.; Fernández de la Mora, J. Submitted to *J. Am. Soc. Mass Spectrom.*

(11) Martínez-Lozano, P. *Proceedings of the 56th ASMS Conference on Mass Spectrometry and Allied Topics*, Denver, CO, June 1–5, 2008; WP 317.

(12) Chen, H.; Wortmann, A.; Zhang, W.; Zenobi, R. *Angew. Chem., Int. Ed.* **2007**, *46*, 580–583.

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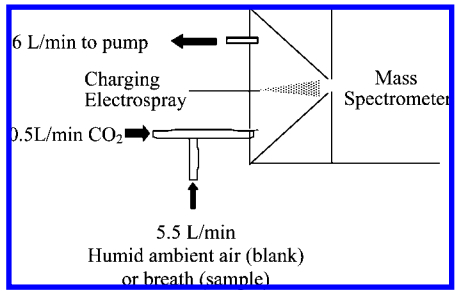

Figure 1. Experimental setup used for the detection of acid vapors present in breath.

hold a closed chamber (Figure 1), which supports an electrospray source facing the MS sampling orifice. This instrument has a mass range of 5−40 000 m/z and a nominal mass resolution of 8 000 (full width at half-maximum, fwhm) at m/z 829 with positive ion MS. Mass accuracy is ~60 ppm. For our range of interest (50−300 Da), the resolution used was ~5 000. Neutral vapors enter the chamber through an inlet tube ($^1/_4$ in. o.d.) and are forced to pass through a negative electrospray (ES) plume. Some of them are ionized by the charged drops (or by solution ions released from the drops) and sucked into the MS to be finally "weighed". A flow rate of 6 L/min is driven in and out of the charging chamber with a pump connected to a second outlet $^1/_4$ in. tube. This flow is a mixture of 0.5 L/min of CO_2 (meant to avoid electrical discharges with negative ES) and 5.5 L/min of either ambient laboratory air or breath. The breath sample is taken by sealing the sampling tube with the lips and letting the pump sample the gas from the lungs without opposing any resistance or forcing it in, so that the pressure is almost identical to atmospheric pressure, and the flow rates of sample and background are also almost the same (as seen directly in the outlet flowmeter). Gloves were worn when handling the sampling tube to avoid contamination coming from the skin. Note that the breathing subject fasted overnight to minimize interferences coming from the mouth.

Exhaled breath consists of vapors previously inhaled from the ambient, with some additions and subtractions made in the lung. Accordingly, one must discriminate between endogenous and exogenous vapors. As illustrated later, we have noted that humidity drastically affects the ionization efficiency of many vapors. Consequently, our blank is based on humidified room air and is taken every time immediately before the breath sample. In this way, by simply substituting humid room air spectra from breath spectra, we preserve the detection probability of background contaminants as well as metabolites. In our experiment, ambient air is humidified upstream of the ionization chamber by passing it through the headspace of a flask containing distilled water at 37 °C. A hygrometer indicated ~96% of relative humidity in the headspace of the flask, a value comparable to the humidity measured when breathing toward the hygrometer. In our previous study in positive ionization,[9] humidity effects were not taken into account, so that some of the peaks associated with breath could have been spurious. The mass spectrometer uses a dry stream of curtain gas flow precluding penetration of neutral vapors into the analyzer, which handled this high humidity with no apparent interference of hydrated peaks.

Note that this technique is online and requires no sample preparation. The sampling tube is continuously drawing room air at a fixed flow rate, and the subject needs only to exhale in it. We

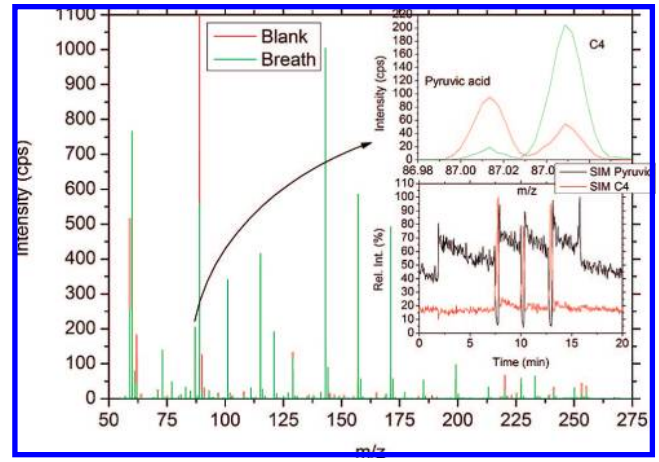

Figure 2. Comparisons of the spectra for the sample and the humid blank. The top inset shows a zoom at 87 Da. The bottom inset shows the time variation of the normalized MS signal for the pyruvate and butanoate (C4) ions when humidifying room air (minute 1.8), as the subject breathes (minutes 7.7, 10.2, and 12.9) and when removing the water flask (minute 15.7).

operated the MS in negative mode and ran an ES of 0.1% NH_4OH in 1:1 MeOH/H_2O (v/v), obtaining in this way deprotonated vapors (molecular weight − 1 Da).

RESULTS AND DISCUSSION

Figure 2 shows two typical mass spectra, one from humidified ambient air and another from breath. The background is much simpler in the negative than in the positive ion mode, and only a few peaks are higher in the background than in breath. The upper inset shows a zoom at m/z = 87 Da, where we can distinguish two peaks. One corresponding to α-ketopropionic acid (pyruvic acid, m/z = 87.0075 Da), the other to butanoic acid (m/z = 87.0442 Da). Their identity was confirmed by comparing collision-induced-dissociation (CID) spectra of both compounds with an online mass spectral database reference (www.massbank.jp). Butanoic acid is clearly more concentrated in breath than in ambient air, while the opposite holds for pyruvic acid. The later observation is unsurprising in a relatively closed environment containing people, since pyruvic acid is a known constituent of skin emanations.[13] A more detailed picture of what is happening can be seen in the single ion monitoring (SIM) measurement shown in the lower inset. One can distinguish several steps. During the first 1.8 min, the mass spectrometer is analyzing air from the laboratory not humidified (RH ~22%). From minutes 1.8 to 15.7 we placed the sampling tube in the headspace of the water flask. At 7.7, 10.2, and 12.9 min, the operator blows on the sampling tube and finally removes the water flask at minute 15.7. In the sequence corresponding to pyruvic acid, we observe a clear increase in the signal (from ~40% to ~70%) when placing the water flask and then a monotonic decrease to ~55%. The sharp initial rise is partly a response to pyruvic acid in the skin of the operator's hand when he manipulates the sampling tube to introduce it in the flask. This effect has been studied in greater detail elsewhere[13] without the complicating effects of humidity. After withdrawing the hand, the signal settles into ~55%, with an increase from the

(13) Martínez-Lozano, P.; Fernández de la Mora J. Submitted to *Rapid Commun. Mass Spectrom.*

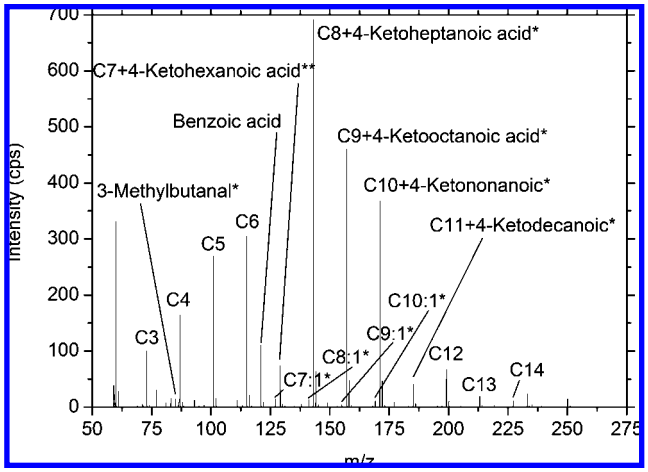

Figure 3. Background-subtracted spectrum based on the data of Figure 2, indicating the deprotonated acids identified. ∗, Not positively confirmed by MS/MS, although the most reasonable identity is listed. ∗∗, The structure is known to a reasonable degree of certainty, but uncertainty exists in the location of the keto group.

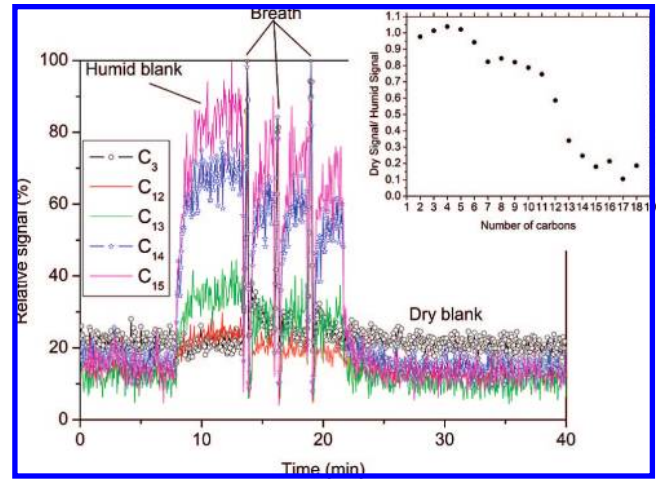

Figure 4. SIM trace for different saturated fatty acids. The inset represents the inverse of the charging probability enhancement due to humidity as a function of chain length.

initial 40% due to the effect of the changed humidity on the ionization efficiency of pyruvic acid. Clearly, the concentration of pyruvic acid in the lung is much lower, as observed at minutes 7.7, 10.2, and 12.9. Again, the signal increases when the operator removes the flask from the system (minute 15.7) and recovers the original level of ~40% from the ambient dry air. The pattern is reversed in the case of butanoic acid (C4), whose concentration is far greater in breath than in ambient air, while humidity seems to have almost no effect.

The heaviest ion seen very clearly above the noise, appears at 250.1438 Da. Interestingly, although its identity remains unknown, it is also observed among the volatiles above urine headspace.[11] The mass spectrum can be simplified by taking the difference between sample and humid background, as shown in Figure 3. Most of the dominant peaks in the spectrum form part of a series spaced by 14 Da, clearly associated to CH_2 addition in a hydrocarbon chain. The series starts with a weak peak at 73 Da, corresponding to deprotonated propionic acid (C3), whose structure was confirmed by its exact mass combined with CID. The series continues uninterrupted with well-defined peaks up to deprotonated tetradecanoic acid (myristic; C14). Along with the fatty acids, the TOF cleanly resolves another series of peaks slightly lighter, starting with pyruvic acid (see upper inset Figure 2) and ending at 185 Da. This series most likely corresponds to ketomonocarboxylic acids, according to their exact mass (besides the positive identification by CID of pyruvic and 4-ketohexanoic acid). Note that the breath signal exceeds the background only from 129 Da (4-ketohexanoic acid) to 185 Da (4-ketodecanoic acid). The particular position of the keto group has not been confirmed by alternative procedures and could be different. Interestingly, 2-ketohexanoic acid is a known potent insulin secretagogue.[14] Benzoic acid (121 Da) is also present, as confirmed by MS/MS. 3-Methylbutanal (85 Da) has been tentatively identified, and some other minor peaks may be also aldehydes according to their exact mass. For instance, butanal (71 Da); 3-methylbut-2-enal (83 Da); 3-hexenal (97 Da; related to α-linolenic acid metabolism); 4-methylpentanal (99 Da); and heptanal (113 Da).

We also observe a series of peaks displaced 2 Da to the left of the main series. On the basis of their exact mass, they correspond most probably to singly unsaturated fatty acids: C7:1 (127 Da), C8:1 (141 Da), C9:1 (155 Da), and C10:1 (169 Da). Larger chains up to C18:1 are observed in the background at concentrations larger than in breath. The dominant background peak from Figure 2 at 89 Da corresponds to 2-hydroxypropanoic acid (lactic acid), secreted in bulk quantities by the skin.[13] However, we observe 2-hydroxyhexanoic acid (131 Da), 2-hydroxyheptanoic acid (145 Da), and 2-hydroxyoctanoic acid (159 Da) clearly above the background level (assigned only by their exact mass).

Effect of Humidity on Ionization Probability. As mentioned earlier, humidity has a great impact on the signal of many background peaks, and this seems to be in accordance with other observations where humidity enhances the signal from electrosprayed ions.[15] Figure 4 displays the SIM trace for C3 and C12−C15. Humidity has almost no effect for the short chain fatty acid C3, whereas it greatly enhanced the signal of the longer fatty acids. Humidity increases the background signal of C15 by a factor of 5.7, while breath decreases it well below the humid blank. The filled symbols in the inset show the ratio between the background fatty acid signal under ambient and humidified conditions. The fatty acid samples used to construct this curve beyond C15 are from the background rather than from breath. A similarly strong effect of water content on ionization efficiency was observed for large singly unsaturated fatty acids from C14:1 to C18:1.

An effect of humidity is to be expected, since the charging ions would tend to solvate more in humid than in dry air and this could affect their reactivity with neutral vapor molecules. Perhaps the polar end of the neutral acid vapor dissolves in (or interacts better with) the widened solvation shell surrounding the solvated charging ions, facilitating their deprotonation reaction (possibly because the nonpolar chain protects the acidic end from reagent ions). Furthermore, evaporation of a few molecules from the

(14) Lenzen, S.; Formanek, H.; Panten, U. *J. Biol. Chem.* **1982**, *257* (12), 6631–6633.

(15) Nguyen, S.; Fenn, J. B. *Proc. Natl. Acad. Sci. U.S.A.* **2007**, *104* (4), 1111–1117.

Table 1. List of Saturated Fatty Acids Observed in Breath and Their Corresponding Water Solubility (At 25 °C), Typical Blood Concentration Values for Healthy Adults, Vapor Pressure, Henry's Constant, and pK_a

no. of carbons	solubility (μM)	concentration in blood (μM)	vapor pressure (Torr)	Henry's constant (μM/Torr)	pK_a
3	4.8×10^6 (predicted by ALOGPS)[34]	0.9 ± 1.2[36]	8.1 (at 37 °C)[38]	7.5×10^{6} [45]	
4	2.7×10^6 (predicted by ALOGPS)[34]	1.0 (0.3−1.5)[36]	2.5 (at 37 °C)[39]	6.18×10^{6} [45]	4.83[16]
5	2.4×10^5 (experimental)[35] 3.9×10^5 (predicted by ALOGPS)[34]	0.6 (0.3−1.2)[36]	5.1×10^{-1} (at 37 °C)[40]	2.89×10^{6} [45]	4.83[16]
6	8.9×10^4 (experimental)[35] 8.4×10^4 (predicted by ALOGPS)[34]	0.8 (0.0−1.6)[36] 17.0 (0.0−105.0)[37]	7.3×10^{-2} (at 37 °C)[41]	1.84×10^{6} [45]	4.85[16]
7	2.2×10^4 (experimental)[35] 2.2×10^4 (predicted by ALOGPS)[34]		2.2×10^{-2} (at 37 °C)[42]		4.89[16]
8	5.5×10^3 (experimental)[35] 6.3×10^3 (predicted by ALOGPS)[34]	2.5 (0.0−5.0)[36] 8.0 (5.0−19.0)[37]	3.7×10^{-3} (at 37 °C)[41]		4.89[16]
9	1.8×10^3 (predicted by ALOGPS)[34]				4.96[16]
10	3.6×10^2 (experimental)[35] 5.5×10^2 (predicted by ALOGPS)[34]	11.0 (5.0−17.0)[37]			
11	2.8×10^2 (experimental)[35] 1.2×10^2 (predicted by ALOGPS)[34]				
12	2.4×10^1 (experimental)[35] 5.0×10^1 (predicted by ALOGPS)[34]	12.0 (2.0−37.0)[37]	1.2×10^{-4} (at 37 °C)[43]		4.85[17]
13	1.8×10^1 (predicted by ALOGPS)[34]		1.1×10^{-5} (at 25 °C)[44]		
14	4.7 (experimental)[35] 7.5 (predicted by ALOGPS)[34]	2.1 ± 0.9[36] 25.0 (8.0−70.0)[37]	2.4×10^{-6} (at 25 °C)[44]		4.89[18]

solvation nanodrop would provide an additional channel to release heat received from the charge exchange reaction. Alternatively, the charge exchange reaction of solvated ions may involve release of solvent vapor, whereby an increased vapor concentration would modify not only the kinetics but also the equilibrium composition. Conversely, Fenn and colleagues[15] have observed considerable enhancement of ionization rates from analytes dissolved in electrospray drops evaporating in slightly humid environments. They have proposed that the effect is due to local heat released by individual vapor molecule condensation events on the electrospray drops.

The observation that the enhancement of the ionization probability due to humidity appears to approach a plateau at C15 provides some clues on the underlying mechanism. It is unlikely to be connected to acid dissociation in solution, since the pK_a in water reaches a plateau with chain length at much smaller chain length than observed for the gas phase ionization probability (pK_a = 3.75,[16] 4.756,[16] 4.83,[16] 4.83,[16] 4.85,[16] 4.89,[16] 4.89,[16] 4.96,[16] 4.85,[17] and 4.89[18] for formic, acetic, butanoic, pentanoic, hexanoic, heptanoic, octanoic, nonanoic, dodecanoic, and tetradecanoic acids, respectively. See also Table 1).

Although a rigorous explanation of the mechanism of humidity-enhanced charge exchange is beyond the scope of this study, it shows that background correction in breath analysis is not trivial.

Figure 5 compares the different traces for 4-ketohexanoic acid (129.0550 Da), C7 (129.0904 Da), and the heaviest peak observed at 250.1438 Da. On the left we can see the considerable, modest, and negligible effect of humidity on 4-ketohexanoic acid, C7, and the peak at 250 Da, respectively. Note also that the subject breathes only during the rising part of the black curve (~8 s; ~0.7 L), while its descending piece (and the still ascending ones from the blue and red traces) are associated to the response time of the system. Interestingly, the whole series of ketomonocarboxylic acids presents a similar response. This suggests a different memory effect depending on the nature of the ion. Note that the system was not heated, which may explain this long response time. Known differences between the intraoral and the alveolar (the last to emerge in breath) parts of breath[19] could also provide cues to rationalize such different behaviors.

Online Detection of Fatty Acids. This is the first report of which we are aware of a mass spectrum with such a wide group of fatty acids obtained online directly from breath vapors. Many prior studies have observed fatty acids by less direct and generally much slower techniques. Fatty acids are known to be an important component of skin secretions,[20,21] feces,[22] and urine.[22] Probably for this reason, they also play signaling roles in a diversity of species. For instance, fatty acids are also important in prey detection by the female mosquito,[21,23] which are capable of sensing vapor sources of fatty acids at least as heavy as C18.[23]

The sensitivities to fatty acid vapors attained in prior studies have varied widely depending on the analytical methods used. For instance, the largest component detected by so-called SIFT

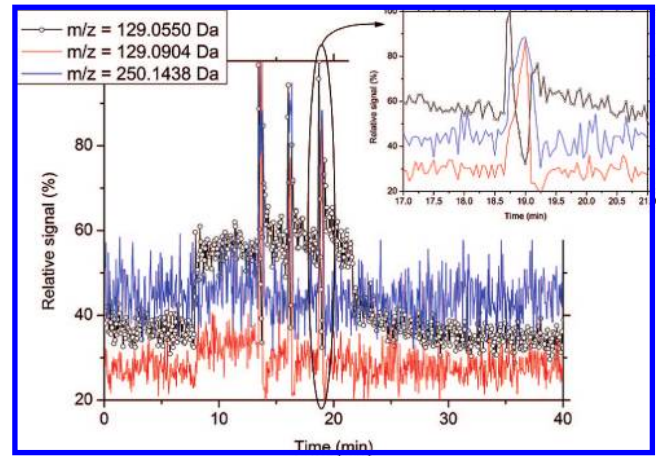

Figure 5. SIM traces for 2-ketohexanoic acid (129.0550 Da), C7 (129.0904 Da), and the heaviest peak observed at 250 Da.

(16) Lide, D. R. In *CRC Handbook of Chemistry and Physics*, Internet Version 2005, ed.; http://www.hbcpnetbase.com, CRC Press: Boca Raton, FL, 2005.
(17) Beckett, A. H.; Moffat, A. C. *J. Pharm. Pharmacol.* **1969**, *21*, 144S–150S.
(18) Taylor, M. A.; Princen, L. H. In *Fatty Acids. Fatty Acids in Solution*; Pryde, E. H., Ed.; The American Oil Chemists' Society: Champaign, IL, 1979; pp 195−217.

MS in the headspace above fecal samples from swine was hexanoic acid, at concentrations from 55 000 to 250 000 ppt.[22] The ions formed were positively charged by attachment of H_3O^+, rather than deprotonated as here. Mills and Walker[24] have used solid phase microextraction of headspace from urine samples followed by GC/MS, (a collecting fiber was exposed to urine vapor for 30 min at 50 °C and desorbed for 2 min). Even with this sample concentration scheme and their elevated temperature operation, they were able to detect only up to nonanoic acid. Far more impressive results have been obtained from condensed sweat samples heated and concentrated at liquid nitrogen temperature (cryofocusing) for 10 min before analysis by GC/MS.[21] This study reports that the majority of the intense peaks observed in the chromatogram are carboxylic acids, dominated by hexadecanoic and octadecanoic acids.

One key advantage of our direct online method of analysis of breath vapor is its speed and simplicity. For instance, a typical breath analysis by the dominating GC/MS technique[25] requires sample collection, usually onto a sorbent, which takes about 5 min. Subsequent GC/MS analysis typically requires some 25 min. This procedure should be repeated for the analysis of the background air, providing a total time in the range of ~1 h. In the case proposed here, the background air and breath are monitored continuously, as shown in Figures 4 and 5. A typical ES-MS analysis requires some 30 s for the analysis of the ambient air and another ~30 s for breath. Thus, the estimation of time saving is in the range of a factor of 60.

It is also more sensitive than most prior approaches involving condensed sample collection, volatilization, concentration in a trap, and then GC/MS analysis. Nevertheless, we should keep in mind some limitations of this technique. For example, its inability to sense nonpolar species such as alkanes. Also, the background interference in breath analysis is a well-known problem.[25] In our case, it is convenient to use noble materials (i.e., stainless steel) on the manufacturing of the ionization chamber. For the case of chemical noise originating primarily from the individuals present in the room (as the case of fatty acids), it seems reasonable to hypothesize that it can be minimized upon ventilation of the room. Independent of the origin of the background, we have followed the blank-subtraction approach, commonly used in GC/MS,[25] which allows discrimination between endogenous and exogenous species. Another peculiarity to be taken into account, especially if one means to pursue quantitative analysis, is the effect of the humidity on the ionization probability mentioned above.

Quantification of Fatty Acid Concentration in Breath. The higher concentration of acid vapors we observe in breath versus background must be attributed to addition of vapor in the lung resulting from vapor exchange between solutes in the blood and the gas phase. Fatty acids dissolved in blood, like O_2 and CO_2, reach equilibrium with the gas at the lung. Expected breath concentrations can therefore be inferred from the blood mean concentration and Henry's law constant, which provide a measure of the partition of a substance between the atmosphere and the aqueous phase. Corresponding data are provided in Table 1 for a few representative acids, with associated expected gas phase concentrations represented as ● in Figure 6. In order to infer gas phase concentrations from the experimental mass spectrometric signal, we provisionally assume that charging and transmission probabilities within the MS are similar to those measured for TNT vapor (in the same instrument using a similar charging scheme leading also to deprotonation of TNT),[10] which gave 3 ions/s at a concentration of 1.1 ppt. This corresponds to 21 ppt of tetradecanoic acid in the breath sample. The ○ symbols of Figure 6 display the corresponding experimental concentration for C3−C6, showing values comparable to those expected from the blood concentrations and Henry's constant. Note in Table 1 the low fatty acid concentrations in blood relative to saturation levels for all but the heavier ones listed. The activity coefficients (ratio between the vapor pressure of a real and an ideal solution) also rise high above unity with increasing chain length (~413 for C6). Hence, breath concentration is initially not limited by solubility, and when it begins to be so limited (at ~C14), its value is substantially higher than would be expected from vapor pressure data for ideal solutions. The data in Table 1 imply a vapor pressure for C14 of $\sim 1.4 \times 10^{-3}$ ppt for an ideal solution (~0.6 ppt if it had the same activity coefficient as C6), compared to a value of 21 ppt inferred from measured MS abundance. The system therefore appears to have sufficient sensitivity to detect even heavier fatty acids, were it not for the increased competition from cutaneous background.

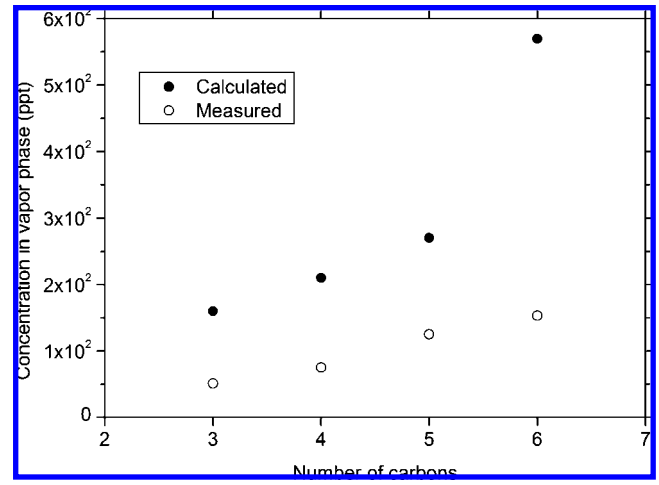

Figure 6. Representation of calculated vapor phase concentration based on typical blood concentrations and Henry's constant and the inferred concentrations measured when breathing for C3−C6. For reference, Table 1 lists solubility, blood concentrations for healthy subjects, and vapor pressures of saturated carboxylic acids observed in breath in this study.

Relevance of Fatty Acids to Medical Diagnosis. There is a considerable breath-related literature linking fatty acid metabolism to various diseases, which, although based on relatively slow measurement protocols, suggests their importance as biomarkers. Fatty acids are generally not directly measured in GC/MS studies,

(19) Van den Velde, S.; Quirynen, M.; Van Hee, P.; Van Steenberghe, D. *Anal. Chem.* **2007**, *79*, 3425–3429.
(20) Curran, A. M.; Rabin, S. I.; Prada, P. A.; Furton, K. G. J. *J. Chem. Ecol.* **2005**, *31* (7), 1607–1619.
(21) Bernier, U. R.; Kline, D. L.; Barnard, D. R.; Schreck, C. E.; Yost, R. A. *Anal. Chem.* **2000**, *72*, 747–756.
(22) Smith, D.; Španěl, P.; Jones, J. B. *Bioresource Technol.* **2000**, *75*, 27–33.
(23) Bosch, O. J.; Geier, M.; Boeckh, J. *Chem. Senses* **2000**, *25* (3), 323–330.
(24) Mills, G. A.; Walker, V. *J. Chromatogr., B* **2001**, *753* (2), 259–268.
(25) Phillips, M. *Anal. Biochem.* **1997**, *247*, 272–278.
(26) Novak, B. J.; Blake, D. R.; Meinardi, S.; Rowland, F. S.; Pontello, A.; Cooper, D. M.; Galassetti, P. R. *Proc. Natl. Acad. Sci. U.S.A.* **2007**, *104* (40), 15613–15618.

but their potential value to monitor hyperglycemia episodes in diabetic patients has been previously suggested.[26] There is also considerable literature based on analysis of radioactive $^{13}CO_2$ or DO_2 in breath, following ingestion of deuterium or ^{13}C labeled fatty acids.[27−30] Also of interest is so-called exhaled breath condensate (EBC) analysis, where the subject breathes into a cooled tube, where exhaled condensable species are trapped and accumulated for about 20−30 min. This material (mostly water) is then thawed, collected into a vial, and analyzed, generally via liquid chromatography−MS. Surprisingly, involatile substances such as proteins, DNA, 8-isoprostane, etc., are collected by an unknown mechanism.[31,32] One recent EBC study[33] reports the observation of increased malondialdehyde levels in patients with chronic obstructive pulmonary disease. Interestingly, an ion with exactly the same mass is observed here but is more abundant in the background than in breath. It is not clear from the literature if current EBC practice corrects properly for vapors in the background. The same species has been observed in a study of volatile emissions from the skin of a healthy individual,[13] with an inferred concentration of ∼25 ppt. These and many other related studies suggest the interest of online methods to detect fatty acids.

CONCLUSIONS

API-MS analysis of breath vapors deprotonated by contact with a negatively charged basic electrospray cloud produces many intense peaks, primarily associated with saturated fatty acids and (most probably) unsaturated fatty acids and ketomonocarboxylic acids. A group with minor signals is most probably associated with aldehydes. This approach is online and requires no sample concentration. However, because of the strong effect of water vapor in the ionization probability of many breath vapors and the high humidity of breath, corrections for background concentrations require moisturizing the ambient gas. A semiquantitative analysis for short chain carboxylic acids provides concentrations in the breath of $\sim 10^2$ ppt, comparable to those expected from typical blood concentrations.

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(27) Delaney, W. E.; Nickoloff, E.; Ettinger, D. D. S.; Drew, H. N. M.; Szeluga, D. J.; Stuart, D. R. K. *Cancer* **1982**, *503*, 727–731.
(28) Maes, B. D.; Geypens, B. Y. J.; Ghoos, Y. F.; Hiele, M. I.; Rutgeerts, P. J. *Gastroenterology* **1998**, *114*, 856–863.
(29) Votruba, S. B.; Zeddun, S. M.; Schoeller, D. A. *Int. J. Obesity* **2001**, *25*, 1240–1245.
(30) McCloy, U.; Ryan, M. A.; Pencharz, P. B.; Ross, R. J.; Cunnane, S. C. *J. Lipid Res.* **2004**, *45*, 474–485.
(31) Horváth, I.; Hunt, J.; Barnes, P. J. *Eur. Respir. J.* **2005**, *26*, 523–548.
(32) Kharitonov, S. A.; Barnes, P. J. *Proc. Am. Thorac. Soc.* **2004**, *1*, 191–199.
(33) Corradi, M.; Rubinstein, I.; Andreoli, R.; Manini, P.; Caglieri, A.; Poli, D.; Alinovi, R.; Mutti, A. *Am. J. Respir. Crit. Care Med.* **2003**, *167*, 1380–1386.
(34) Tetko, I. V.; Gasteiger, J.; Todeschini, R.; Mauri, A.; Livingstone, D.; Ertl, P.; Palyulin, V. A.; Radchenko, E. V.; Zefirov, N. S.; Makarenko, A. S.; Tanchuk, V. Y.; Prokopenko, V. V. *J. Comput.-Aided Mol. Des.* **2005**, *19*, 453−463, http://www.vcclab.org.
(35) Yalkowsky, S. H.; Dannenfelser, R. M. In *Aquasol Database of Aqueous Solubility*, version 5; College of Pharmacy, University of Arizona: Tucson, AZ, PC version, 1992.
(36) *Geigy Scientific Tables,* ; 8th rev. ed.; Lentner, C., Ed.; : Medical Education Divsion, Ciba-Geigy Corp.: West Cadwell, NJ and Basel, Switzerland, 1981−1992; pp 165−177.
(37) Hoffmann, G. F.; Meier-Augenstein, W.; Stockler, S.; Surtees, R.; Rating, D.; Nyhan, W. L. *J. Inherited Metab. Dis.* **1993**, *16* (4), 648–669.
(38) Dreisbach, R. R.; Shrader, S. A. *Ind. Eng. Chem.* **1949**, *41*, 2879–2880.
(39) Jasper, J. J.; Miller, G. B. *J. Phys. Chem.* **1955**, *59*, 441–442.
(40) Kahlbaum, G. W. A. *Ber. Dtsch. Chem. Ges.* **1883**, *16*, 2476–2484.
(41) Rose, A.; Acciari, J. A.; Johnson, C.; Sanders, W. W. *Ind. Eng. Chem.* **1957**, *49* (1), 104–109.
(42) Stull, D. R. *Ind. Eng. Chem.* **1947**, *39*, 517–540.
(43) Hammer, E.; Lydersen, A. L. *Chem. Eng. Sci.* **1957**, *7*, 66–72.
(44) Chattopadhyay, S.; Ziemann, P. J. *Aerosol Sci. Technol.* **2005**, *39* (11), 1085–1100.
(45) Khan, I.; Brimblecombe, P.; Clegg, S. L. *J. Atmos. Chem.* **1995**, *22*, 285–302.