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Analysis of the isobaric compounds propanol, acetic acid and methyl formate in humid air and breath by selected ion flow tube mass spectrometry, SIFT-MS

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ABSTRACT

Numerous analyses of exhaled breath using selected ion flow tube mass spectrometry, SIFT-MS, over the last few years have revealed the presence of volatile compounds with molecular weight 60 and the concern has been to identify which of the isobaric compounds from the set of 1-propanol, 2-propanol, acetic acid and methyl formate are present in human breath. The problem is compounded by the formation of hydrates of the characteristic primary product ions of the reactions of the H_3O^+ and NO^+ precursor ions with these compounds, this being particularly efficient for humid samples such as exhaled breath. Thus, the resulting product ion spectra are complex and choices have to be made as to which of the characteristic product ions and their hydrates can best be used for the quantitative analyses. To facilitate this choice for the particular problem of identifying and quantifying the four aforementioned isobaric compounds, a study has been made of the ion chemistry of H_3O^+ and NO^+ with the two propanol isomers, acetic acid and methyl formate for increasing sample humidity up to that of exhaled breath, which is about 6% by volume. The problems involved in the separate analysis of propanol have been met and solved by previous SIFT-MS studies and now the present study has revealed how acetic acid and methyl formate can be separately identified in a humid mixture using NO^+ precursor ions only. Following this work, the kinetics database entries for the SIFT-MS analyses of these compounds in breath have been constructed and the analysis of the exhaled breath of five healthy volunteers showed that, in addition to the propanol isomers, acetic acid was present at levels typically within the range from 30 to 60 parts-per-billion by volume and that methyl formate was not present above the limit of detection.

1. Introduction

The identification and quantification of isobaric compounds, especially when they are present at low levels in a gas mixture as complex as exhaled breath, is a serious challenge that has to be met if accurate analyses of such compounds are to be realised using selected ion flow tube mass spectrometry, SIFT-MS. The classic example of such compounds is the aldehydes and ketones, which are ubiquitous in nature and are certainly present in human breath [1–4]. We have had to be very vigilant during the development of SIFT-MS for the study of exhaled breath to avoid the trap of wrongly identifying ketones as aldehydes and *vice versa*, since both of these compounds are readily protonated by H_3O^+ ions that are commonly used as the precursor (reagent) ions in SIFT-MS analyses [5]. Thus, isobaric compounds such as acetone and propanal with the same molecular weight, *M*, form primary product ions MH^+ [6] obviously having the same mass-to-charge ratio, m/z, that cannot be separated by the analytical quadrupole mass spectrometer. However, the other available precursor ions in SIFT-MS now come into use, especially NO^+, since their reactions can often be used to distinguish between isobaric compounds. For example, the NO^+/ketone reactions usually result in NO^+M adducts ions, whereas NO^+/aldehyde reactions usually proceed via hydride ion, H^-, transfer forming $(M-H)^+$ product ions [6,7]. From the inception of SIFT-MS we have been continuously studying the reactions of H_3O^+, NO^+ and also ${O_2}^+$ (also available as a precursor ion in SIFT-MS) with a wide variety of organic and inorganic compounds to provide and upgrade the kinetics library required for trace gas analyses by SIFT-MS. The details of these kinetics studies are given in numerous papers as referenced in a relatively recent review paper [8]. Recently, a similar approach has been taken by using NO^+ in a modified PTR-MS instrument to differentiate between aldehydes and ketones [9].

A related problem has recently arisen during a study of the breath of several patients, in which inspection of the product ion spectra obtained by SIFT-MS analyses revealed the presence in the

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breath samples of one or more volatile compounds with molecular weight 60. The candidates for this compound are the alcohol isomers 1-propanol and 2-propanol, the carboxylic acetic acid and the ester methyl formate, and the problem is to identify using SIFT-MS which of these compounds are present. Indeed, the exhaled breath might contain a mixture of all four compounds! To solve this problem it is essential to identify the products of the reactions of the four candidate compounds with the most useful precursor ions H_3O^+ and NO^+ and, for accurate analyses, determine the rate coefficients for the analytical ion–molecule reactions under the operating conditions of the SIFT-MS instrument. The rate coefficients for ion–molecule association reactions are well known to be dependent on the pressure of the background gas in which the reactions proceed (in this case a mixture of humid air and helium) and this is the situation for the NO^+ reactions with acetic acid and methyl formate. These reactions result in the adduct ions NO^+CH_3COOH and $NO^+HCOOCH_3$, as shown by an early SIFT study carried out at a helium carrier gas pressure of 0.5 Torr [10] which, significantly, is lower than the pressure at which the current SIFT-MS instruments operate of typically 1 Torr [11]. Further to this, the ion products of the reactions of both H_3O^+ and NO^+ with most compounds can include the hydrates of the products ions, e.g., $MH^+(H_2O)_{1,2}$ and $NO^+M(H_2O)$, when the sample is humid [12], as is the case for exhaled breath, and so all the product ions and ideally the ion chemistry pathways leading to them should be identified under the conditions of the chemical reactor if accurate analyses are to be obtained. This must also include a measurement of the humidity of the sample, as will be seen.

Thus, we have carried out a study of the reactions of H_3O^+ and NO^+ with the isobaric 1-propanol, 2-propanol, acetic acid and methyl formate using the same *Profile 3* SIFT-MS instrument (Instrument Science, Crewe, UK) for these kinetic studies that we now routinely use for trace gas analysis of air and exhaled breath, over a range of sample gas humidity from about 0.5% to about 6% in order to identify the product ion and the hydrate ion distributions and the rate coefficients for the reactions. It will be shown that the product ion data allow the predominant compounds with molecular weight 60 present in breath samples to be identified and the rate coefficients allow their accurate quantification.

1.1. Ion chemistry

The primary ion chemistry involved in the reactions of H_3O^+, NO^+ and $O_2{}^+$ with the two propanol isomers, acetic acid and methyl formate under flow tube conditions and at low sample humidity has been described in previous publications [10,13], but now it is essential to expand the understanding of the secondary reactions with water molecules to prepare the way for a discussion of the more complex ion chemistry that occurs at higher sample humidity (i.e., in exhaled breath samples). At the onset, it is noted that $O_2{}^+$

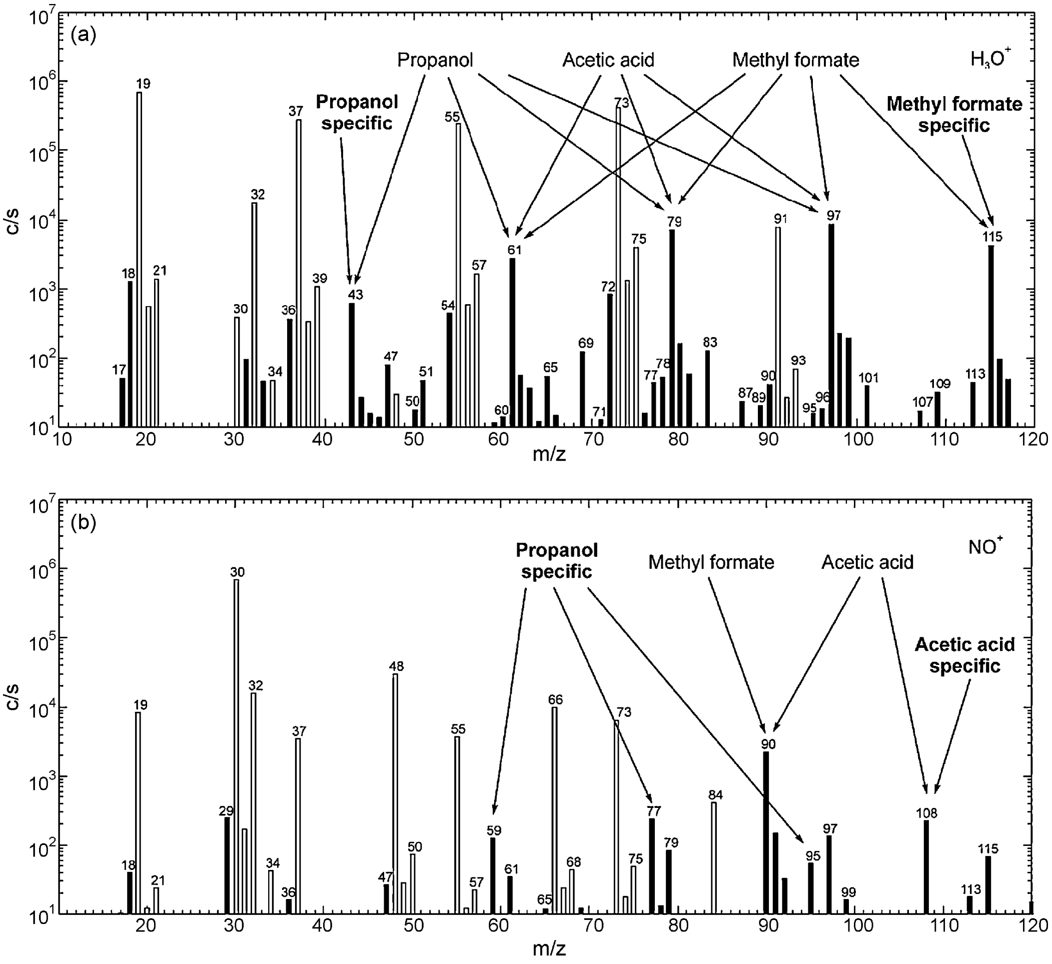

Fig. 1. The full scan spectra obtained using a *Profile 3* SIFT-MS instrument when a mixture of the two propanol isomers, acetic acid and methyl formate in humid air is flowed into the helium carrier gas using (a) H_3O^+ precursor ions and (b) NO^+ precursor ions to analyse the mixture. The open bars are the precursor ions in each spectrum. The major filled bars indicated show the product ions that result from the reactions of the four compounds present in the mixture, including the primary reaction products, which are at *m*/*z* 43 and 61 for H_3O^+ precursor ions and *m*/*z* 59 and 90 for NO^+ precursor ions, and their several hydrate ions. Note that some of the compounds have common product ions and that there are some compound specific product ions, as indicated.

precursor ions are not suitable for SIFT-MS analysis of the above compounds because they result in reactive ion products that complicate analyses, so we will not be concerned here with the details of these O_2^+ reactions, but rather we concentrate on the H_3O^+ and NO^+ ion chemistry. The problem in separating the propanol isomers, acetic acid and methyl formate by SIFT-MS is illustrated by the spectra shown in Fig. 1, which were obtained using H_3O^+ and NO^+ ions to analyse a mixture of all the four isobaric compounds. As indicated, there are overlaps of the characteristic product ions formed although, significantly, there are distinguishing characteristic ions for the two propanol isomers, one for acetic acid and one for methyl formate. These will be referred to again later.

The primary reactions of H_3O^+ and NO^+ with the propanol isomers that occur exclusively at low sample humidity are relatively simple and are summarised thus:

$$H_3O^+ + C_3H_7OH \rightarrow C_3H_9O^+ + H_2O \tag{1a}$$

$$H_3O^+ + C_3H_7OH \rightarrow C_3H_7^+ + 2H_2O \tag{1b}$$

The rate coefficients for both propanol isomer reactions are close to the collisional limiting value and the product ions are the same except there is some difference in the branching ratio in reaction (1) [13,14]. The NO^+ reactions are similarly fast and the product ions are the same for both isomers, hydride ion transfer being the only process [13].

$$NO^+ + C_3H_7OH \rightarrow C_3H_7O^+ + HNO \tag{2}$$

The primary reactions of H_3O^+ with acetic acid and methyl formate proceed at the collisional rate and the single product ions in both reactions is the stable protonated molecule, e.g.:

$$H_3O^+ + CH_3COOH \rightarrow CH_3COOH_2^+ + H_2O \tag{3}$$

However, the reactions of NO^+ with this carboxylic acid and the ester both proceed via relatively slow helium and air mediated three-body association producing the adduct ions, e.g.:

$$NO^+ + HCOOCH_3 + He \rightarrow NO^+HCOOCH_3 + He \tag{4}$$

Thus, in dry sample mixtures of propanol, acetic acid and methyl formate, NO^+ ions can be used to distinguish the propanol isomers, by the characteristic ion at mass-to-charge ratio, m/z, of 59 from acetic acid and methyl formate that both result in the characteristic ion at m/z of 90. However, the last two compounds cannot be separated and analysed by SIFT-MS analyses of dry air samples.

In humid samples the ion chemistry is much richer and complicated, but it turns out that this complex situation provides a means of distinguishing between the acid and the ester. The presence of H_2O molecules in the reaction flow tube (helium/air carrier gas) results in the formation of hydrates of both H_3O^+ and NO^+ via sequences of three-body association reactions [15,16], e.g.:

$$H_3O^+ + H_2O + He \rightarrow H_3O^+H_2O + He \tag{5}$$

Then dihydrate and trihydrate ions $H_3O^+(H_2O)_{2,3}$ can form and these hydrates can undergo ligand switching reactions with polar molecules, M, producing ions like $MH^+(H_2O)_{1,2}$. The latter ions can also result from three-body association reactions of the MH^+ ions formed in reactions such as (1) and (3). Similar association reactions occur between NO^+ ions and H_2O molecules:

$$NO^+ + H_2O + He \rightarrow NO^+H_2O + He \tag{6}$$

These reactions result in the $NO^+(H_2O)_{1,2}$ ions that are so evident when analysing humid samples using NO^+ precursor ions in SIFT-MS [16]. As above, these adduct ions can react with M to produce ions like NO^+M and NO^+MH_2O and the latter ion turns out to be the key to distinguishing between acetic acid and methyl formate in humid samples, as we will show.

2. Experimental results

As previously stated, the rate coefficients, and the product ion distributions for the reactions of the SIFT-MS precursor ions with the two propanol isomers, acetic acid and methyl formate have been determined previously in SIFT experiments at low humidity and at a helium carrier gas pressure of 0.5 Torr [10,13]. The data obtained in these studies are given in Table 1. These measurements needed to be repeated under the conditions of the analytical *Profile 3* instrument (helium carrier gas pressure 1 Torr, air sample flow rate 0.35 Torr L/s in the Keele instrument used for the present study) and also for sample humidities that correspond to exhaled breath. The well established experimental method to study the kinetics of ion molecule reactions was used, which is described in numerous previous publications including [6–8,10,13,17]. So it is sufficient to just very briefly describe the specific experiments that were carried out to obtain the results presented in this section.

2.1. Rate coefficients

First, the injection quadrupole of the SIFT-MS instrument was set to operate in the total ion mode thus allowing currents of the major ion source (precursor) ions H_3O^+, NO^+ and O_2^+ to be simultaneously injected into the helium carrier gas. A Nalophan bag inflated with dry cylinder air located in a temperature variable enclosure set at 40 °C is connected to the input of the SIFT-MS instrument and then a drop of one of the (volatile liquid) compounds was introduced into the bag. The analytical quadrupole mass spectrometer was set to operate in the multiple ion monitoring (MIM) mode. As the temperature of the bag and contents increased, the flow rate of the vapour into the helium carrier gas increased whilst the flow rate of air was maintained constant. Ultimately, the concentration of the vapour reached a sufficiently high value to reduce the precursor ion count rates at the downstream mass spectrometer detection system by an order-of-magnitude or more. Examples of the decay curves obtained are shown in Fig. 2 for the reactions of the H_3O^+ and NO^+ precursor ions and the $NO^+(H_2O)_{1,2}$ hydrated ions with acetic acid (actually for a humid sample; see below), which shows the relatively slow decay rate of the NO^+ ions. The significance of the faster decay rates of the $NO^+(H_2O)_{1,2}$ ions is discussed below. The slopes of the lines provide the relative rate coefficients for the reactions, k. Note that the k for the H_3O^+ proton transfer reactions is assumed to be equal to the collisional rate coefficients, k_c, calculated using the formulation given by Su and Chesnavich [18], a procedure that has been described in detail in previous papers. In the same way, the k values for the reactions of H_3O^+ and NO^+ with the other three isobaric compounds were determined and these are given in Table 1.

In a second experiment for each compound, first liquid water was injected into the Nalophan bag held at 40 °C and the humidity of the sample gradually increased to the saturated vapour pressure of water, which is about 6% at this temperature (as measured using SIFT-MS [15]). Then, as before, a drop of the liquid compound was introduced into the humid air in the bag and the resulting decay of the precursor ions was monitored. Thus, the rate coefficients for the reactions were obtained under humid conditions approximating to exhaled breath and these are also given in Table 1. We have shown in a recent paper [19] that the CO_2 present in exhaled breath does not interfere significantly with the ion chemistry involving H_2O molecules that is exploited to determine the sample humidity [15].

Note again that the k values for the H_3O^+ reactions included in Table 1 for all four compounds are the respective calculated collisional values, k_c, and these are the reference values for the NO^+ reactions. Inspection of the k values for the NO^+ reactions reveals that the propanol reactions proceed at or very close to their respec-

Table 1
The collisional rate coefficients, k_c, calculated for the reactions of H_3O^+ and NO^+ with the four compounds listed using the formulation given in ref. [18], are given in the third and sixth columns in square brackets. The experimentally derived rate coefficients, k, for the NO^+ reactions at two gas sample humidities (1% and 6%) are also given in the sixth column along with those previously obtained at lower helium pressure and low humidity in round brackets [10,13]. In the fifth and eighth columns are the percent product distributions for the reactions at the two sample humidities.

Molecule	Water vapour concentration (%)	H_3O^+			NO^+		
		[k_c] (10^{-9} cm^3 s^{-1})	Products		[k_c], k (10^{-9} cm^3 s^{-1})	Products	
1-Propanol C_3H_7OH	1	[2.7]	$C_3H_7^+$	(72)	[2.3] 2.0 (2.3)	$C_3H_7O^+$	(50)
			$C_3H_7OH_2^+$	(8)		$C_3H_7O^+H_2O$	(40)
			$C_3H_7OH_2^+H_2O$	(10)		$C_3H_7O^+(H_2O)_2$	(8)
			$C_3H_7OH_2^+(H_2O)_2$	(10)		$C_3H_7O^+(H_2O)_3$	(2)
	6	[2.7]	$C_3H_7^+$	(40)	[2.3] 2.3	$C_3H_7O^+$	(12)
			$C_3H_7OH_2^+$	(2)		$C_3H_7O^+H_2O$	(40)
			$C_3H_7OH_2^+H_2O$	(8)		$C_3H_7O^+(H_2O)_2$	(30)
			$C_3H_7OH_2^+(H_2O)_2$	(48)		$C_3H_7O^+(H_2O)_3$	(18)
			$C_3H_7OH_2^+(H_2O)_3$	(2)			
2-Propanol C_3H_7OH	1	[2.7]	$C_3H_7^+$	(56)	[2.3] 2.3 (2.4)	$C_3H_7O^+$	(76)
			$C_3H_7OH_2^+$	(16)		$C_3H_7O^+H_2O$	(22)
			$C_3H_7OH_2^+H_2O$	(18)		$C_3H_7O^+(H_2O)_2$	(2)
			$C_3H_7OH_2^+(H_2O)_2$	(10)			
	6	[2.7]	$C_3H_7^+$	(24)	[2.3] 2.5	$C_3H_7O^+$	(26)
			$C_3H_7OH_2^+$	(2)		$C_3H_7O^+H_2O$	(62)
			$C_3H_7OH_2^+H_2O$	(12)		$C_3H_7O^+(H_2O)_2$	(12)
			$C_3H_7OH_2^+(H_2O)_2$	(60)			
			$C_3H_7OH_2^+(H_2O)_3$	(2)			
Acetic acid CH_3COOH	1	[2.6]	$CH_3COOH_2^+$	(62)	[2.2] 1.0 (0.9)	NO^+CH_3COOH	(80)
			$CH_3COOH_2^+H_2O$	(28)		$NO^+H_2OCH_3COOH$	(20)
			$CH_3COOH_2^+(H_2O)_2$	(10)			
	6	[2.6]	$CH_3COOH_2^+$	(12)	[2.2] 1.2	NO^+CH_3COOH	(55)
			$CH_3COOH_2^+H_2O$	(20)		$NO^+H_2OCH_3COOH$	(45)
			$CH_3COOH_2^+(H_2O)_2$	(68)			
Methyl formate $HCOOCH_3$	1	[2.7]	$HCOOCH_3H^+$	(66)	[2.3] 0.8 (0.5)	$NO^+HCOOCH_3$	(100)
			$HCOOCH_3H^+H_2O$	(30)			
			$HCOOCH_3H^+(H_2O)_2$	(4)			
	6	[2.7]	$HCOOCH_3H^+$	(12)	[2.3] 1.0	$NO^+HCOOCH_3$	(>98)
			$HCOOCH_3H^+H_2O$	(40)		$NO^+H_2OHCOOCH_3$	(<2)
			$HCOOCH_3H^+(H_2O)_2$	(28)			
			$HCOOCH_3H^+(H_2O)_3$	(20)			

tive k_c values (given in square brackets), which conforms with the previous SIFT measurements for low humidity samples that are given in the round brackets. However, the k values for the NO^+ reactions with acetic acid and methyl formate reactions are obviously lower than their respective k_c values; this is typical of association reactions that form the adduct ions (e.g., reaction (4)). The measured k values in the present study for these reactions are somewhat higher than those obtained in the previous SIFT study under similar low sample humidity [10], principally because of the higher helium carrier gas pressure that promotes the association reactions. At the higher sample humidity the k values for both the acid and the ester reactions are even higher by a factor of 1.2, k for the methyl formate reaction being a factor of 2 greater than that obtained for this reaction under the lower helium pressure and low humidity conditions of the earlier SIFT studies [10]. Such large differences would result in serious errors of quantification in SIFT-MS and this forcibly demonstrates that the rates of ion molecule association reactions are usually dependent on the conditions under which they occur. For such reactions the published k values must be treated with circumspection if accurate SIFT-MS analyses are to be obtained.

Concerning the decay of the $NO^+(H_2O)_{1,2}$ signals shown in Fig. 2. Clearly, the apparent rate coefficients are greater than that for the NO^+ reaction with acetic acid. The signals of these hydrated NO^+ ions will decay as the NO^+ signal decays, since the latter is their precursor ion. However, that the decay rate is faster, and indeed close to the collisional decay rate implies additional loss processes and these are the ligand switching reactions with acetic acid molecules mentioned in the ion chemistry section:

$$NO^+(H_2O) + CH_3COOH \rightarrow NO^+CH_3COOH + H_2O \quad (7)$$

$$NO^+(H_2O)_2 + CH_3COOH \rightarrow NO^+CH_3COOH(H_2O) + H_2O \quad (8)$$

These reactions are automatically included in the SIFT-MS kinetics database used for the analysis, but the signal levels of these

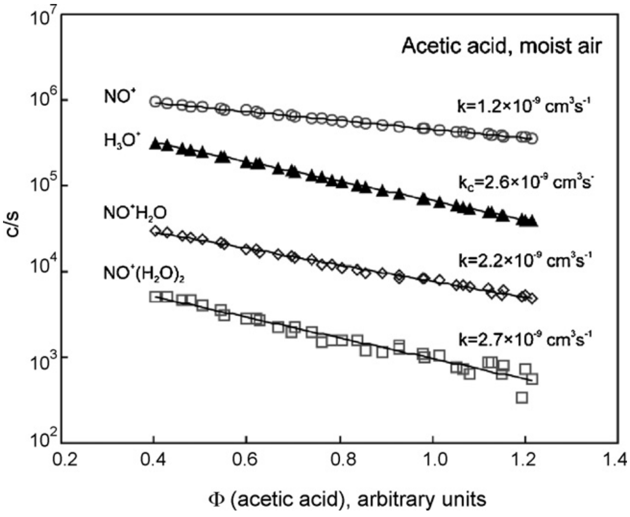

Fig. 2. Plots of count rates per second, c/s, (log scale) of the ions H_3O^+, NO^+, NO^+H_2O and $NO^+(H_2O)_2$ as a function of the flow rate of acetic acid vapour/6% moist air mixture, Φ (in arbitrary units), into the helium carrier gas of the *Profile 3* SIFT-MS instrument. The decay of the H_3O^+ is assumed to be exponential and the rate coefficient is taken as the collisional rate coefficient (see Table 1). The relative slopes of the lines for the other ions provide estimates of the rate coefficients, k, for their reactions with acetic acid as indicated (but see the text).

hydrated ions are relatively small and so the contribution of reactions (7) and (8) to the production of the ions at *m*/*z* values of 90 and 108 is small, the major source being the association reactions of NO^+ with CH_3COOH giving *m*/*z* 90 and the sequential association reaction of NO^+CH_3COOH with H_2O giving *m*/*z* 108.

2.2. Ion product distributions

To identify the product ions of the reaction of chosen precursor ion with a particular neutral compound, a full scan (FS) mass spectrum like those shown in Fig. 1 is routinely acquired as an air/compound vapour mixture is flowed into the system. Ideally, this needs to be done for samples of varying humidity to ensure that all hydrated product ions have been recognised. Then, by targeting the observed product ions using the MIM mode of the SIFT-MS instrument, accurate product ion distributions can be obtained [8]. This requires that the product ion intensities are corrected for mass discrimination and differential diffusion [20–22]. As mentioned in Section 1, using H_3O^+ precursor ions, hydrated ions of the type $MH^+(H_2O)_{1,2}$ form, whereas when using NO^+ adduct ions of the type $NO^+M(H_2O)$ can be formed. In the present study the approach taken to the identification of the product ions as a function of the sample humidity was to place the sample bag containing the dry air/compound mixture into the temperature variable enclosure and to run the SIFT-MS instrument in the MIM mode set to record all the identified products and then to inject liquid water into the bag. The sample humidity increased with time and thus data were obtained for the product ion distributions as a function of the sample humidity.

Such experiments have been carried out previously for the reactions of H_3O^+ and NO^+ with 2-propanol in the context of a study of cancer biomarkers, as reported recently in detail [23], so the similar data obtained in the present study need not be shown in detail. Rather, the product ion distributions for the H_3O^+ reactions with all four compounds included in the present study obtained at only two humidity levels of 1% and 6% are given in Table 1. The corresponding percentages of the H_3O^+ precursor ions and their hydrates at 1% humidity are H_3O^+(86%), $H_3O^+{\cdot}H_2O$ (12%), $H_3O^+{\cdot}(H_2O)_2$ (2%), $H_3O^+{\cdot}(H_2O)_3$ (0%) and at 6% humidity H_3O^+ (42%), $H_3O^+{\cdot}H_2O$ (18%), $H_3O^+{\cdot}(H_2O)_2$ (16%) and $H_3O^+{\cdot}(H_2O)_3$ (24%). Note in Table 1 the several product ions formed in these $H_3O^+(H_2O)_{0,1,2,3}$ reactions and also in the reactions of $NO^+(H_2O)_{0,1,2}$ with the two propanol isomers that proceed initially by hydride ion transfer, as exemplified by reaction (2). Even so, the analysis of propanol in exhaled breath can be achieved using these data with appropriate constructions of the kinetics database library entries [23,22]. The important point here is that the product ion at *m*/*z* 43 seen when the mixture of the four isobaric compounds at MW 60 is analysed using H_3O^+ precursor ions is specific to the propanol isomers, since it is not formed in the acetic acid and methyl formate reactions, and it is on this observation that propanol can be analysed in such mixtures and in exhaled breath that is known to contain both propanol and acetic acid (see later). It is also possible to distinguish propanol from acetic acid and methyl formate using NO^+ ions, since the propanol isomers produce characteristic ions at *m*/*z* 59, 77 and 95 in the analysis of humid samples (see Fig. 1b).

From the product ion ratios given in Table 1 it is possible to estimate the relative contribution of the association reactions of MH^+ with H_2O molecules to the formation of the MH^+ hydrates using a mathematical procedure discussed in detail in ref. [24]. Thus, the association rates for both protonated acetic acid and protonated methyl formate with H_2O are found to be (4 ± 1) times greater than the association rate of H_3O^+ with H_2O that is used in SIFT-MS to determine the water vapour level in samples [15].

The saving grace for distinguishing and analysing acetic acid and methyl formate in complex mixtures such as exhaled breath is the simplicity of the product ions for the reactions of NO^+ with these two compounds, as can be seen in Table 1. At low humidity the only ion products of these reactions are the adduct ions NO^+CH_3COOH and $NO^+HCOOCH_3$ at an *m*/*z* value of 90. However, as the sample humidity is increased the monohydrate ion of the acetic acid adduct appears at an *m*/*z* value of 108, as can be seen in Fig. 3a. Plots of the percentage signal levels of the ions at *m*/*z* values of 90 and 108 as a function of the water level in the air/acetic acid sample gas are shown in Fig. 3b and these data provide the branching ratio for this reaction as a function of the sample humidity. At the humidity appropriate to exhaled breath the branching ratio of *m*/*z* 108 to *m*/*z* 90 is 45:55 (see Table 1). However, very significantly, the monohydrate of the methyl formate ion does not appear under these conditions at signal intensity greater than 2% of the *m*/*z* 90 ion signal. This immediately allows these two isobaric compounds to be distinguished in SIFT-MS and by careful analysis even their relative levels in a complex mixture. Note that the propanol isomers do not form adduct ions with NO^+ ions (see Table 1). An interesting question is why the *m*/*z* 90 acetic acid adduct ion does form a monohydrate whereas that formed by methyl formate does not. A possible explanation is that the structure of the acetic acid adduct is $CH_3CO^+(NO)OH$ that can bind an H_2O molecule as $CH_3CO^+(NO)OH\text{–}OH_2$ whereas no site to which an H_2O molecule

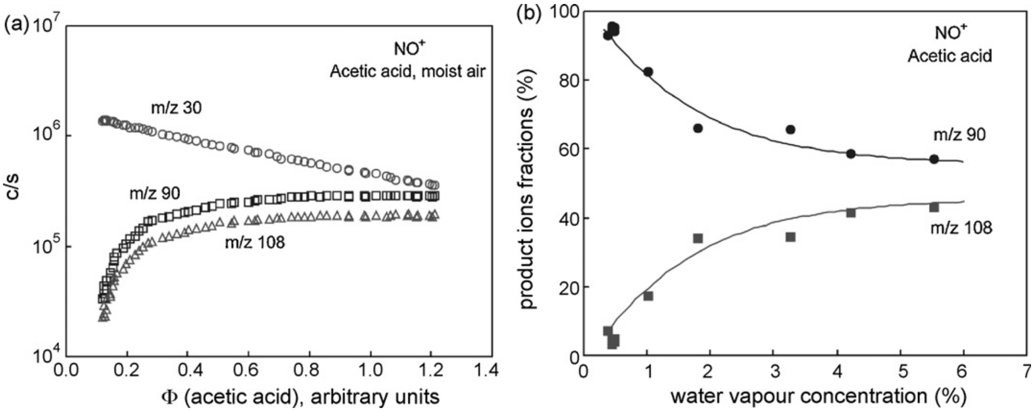

Fig. 3. (a) Plots of count rates per second, c/s, (log scale) of the precursor ion NO^+ and the characteristic product ions of their reaction with acetic acid, NO^+CH_3COOH (*m*/*z* 90) and $NO^+H_2OCH_3COOH$ (*m*/*z* 108), as a function of the flow rate of acetic acid vapour/6% moist air mixture, *Φ* (in arbitrary units), into the helium carrier gas of the *Profile 3* SIFT-MS instrument. (b) The relative signal intensities in percent, %, of the characteristic product ions as a function of the percentage water vapour concentration in the sample mixture.

can readily bind apparently exists in the $HCO^+(NO)OCH_3$ adduct ion.

A final interesting observation regarding methyl formate is that the analysis of this compound in humid samples using H_3O^+ precursor ions in SIFT-MS results in the formation of a trihydrate of protonated methyl formate, $HCOOCH_3H^+(H_2O)_3$, at m/z 115, as can be seen in Fig. 1a. In contrast, as can be seen in Table 1, under identical conditions, product ions at m/z 115 do not form at significant levels above 2% when analysing the propanol isomers and acetic acid. Thus, the m/z 115 product ion is specific to methyl formate, at least to a good approximation. In previous surveys we have found that the formation of a third hydrate of many product ions at 300 K is relatively rare and so far it has only been observed for protonated acetonitrile and protonated nitroalkanes [8]. Only detailed quantum chemical modelling would quantitatively explain why protonated methyl formate readily binds three water molecules whereas protonated acetic acid does not.

2.3. Breath analysis involving compounds at MW 60

As a test for the new database entries for these isobaric compounds, the breath of five healthy volunteers was analysed focusing on the search for propanol, acetic acid and methyl formate. The propanol isomers cannot readily be separated, but the available evidence indicates that breath propanol will largely be the 2-propanol isomer [25]. The breath propanol levels in these three volunteers, as calculated using the kinetic database entry recently constructed for the *Profile 3* SIFT-MS instrument that essentially utilizes only the product ion at m/z 43 with corrections for the presence of protonated propanol at m/z 61 and its monohydrate and dihydrate ions at m/z 79 and 97 [23], were within the range 40–60 parts-per-billion, ppb, which is within the expected range as established by population studies [4]. This analytical approach for propanol avoids complications due to the presence of any acetic acid and methyl formate.

To obtain estimates of the levels of acetic acid and methyl formate in the breath, the relative levels of product ions at m/z values 90 and 108 can be exploited when using NO^+ precursor ions, remembering that m/z 108 ions are not formed from methyl formate (see Table 1 and the above discussion). A significant signal at m/z 108 is evident when analysing the breath of all five volunteers, indicating that acetic acid is present. A plot of the signal level of m/z 108 against the sum of the signal levels at m/z 90 and 108 obtained from these breath analyses is given in Fig. 4. Note that the slope of this line is close to 0.45, i.e., the same as the branching ratio obtained from the experiments with pure acetic acid and humid samples, as shown in Fig. 3b. This is powerful evidence that the breath compound is largely acetic acid and not methyl formate. Additional support for this conclusion comes from the fact that in the reaction of H_3O^+ with pure methyl formate a product ion at m/z 115 appears (see Fig. 1a) and this product ion did not appear in the analyses of the breath of these few volunteers. So these studies show that acetic acid is certainly present in the mouth exhaled breath of healthy individuals. Note that acetic acid has been detected in exhaled breath condensate [26] and in saliva [27] and that a whole series of volatile fatty acids has been detected using electrospray ionisation of breath samples [28].

A question that must be posed in the current phase of development of breath analysis is "what is the origin of trace gases exhaled through the mouth". To assist in answering this question we have carried out measurements of several trace gas compounds in mouth exhaled and nose exhaled breath and in the static gas in the oral cavity [3]. These measurements reveal that some compounds are totally systemic (e.g., acetone, methanol, isoprene) and others are largely generated in the oral cavity by bacterial and/or enzymatic activity (e.g., ammonia, ethanol). The same approach has been taken in the present study and it is found that the oral cavity is definitely a source of acetic acid, but apparently this compound is also partially systemic, i.e., originating at the alveolar interface, since it is present in nose exhaled breath. However, from these observations the partial release of acetic acid from the airways mucosa cannot be ruled out. These preliminary studies indicate that the levels of acetic acid exhaled via the mouth for the five volunteers involved in this study varied from about 30–60 ppb, as obtained using the kinetic data obtained in this study, and that the levels in nose exhaled breath were about a factor of two lower. That the oral cavity is a significant source of acetic acid was also demonstrated by analysing the oral cavity static gas after a mouth wash with water, when the levels were clearly lower at about 10 ppb.

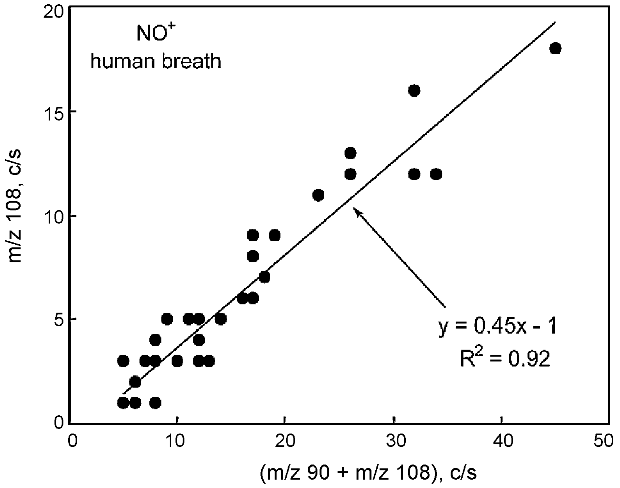

Fig. 4. Plot of count rate per second, c/s, of m/z 108 product ion against the sum of those for the m/z 90 and 108 product ions obtained using the multiple ion monitoring (MIM) mode of SIFT-MS for the analysis of the exhaled breath of five healthy volunteers using NO^+ precursor ions. The slope of the line is in good agreement with the product ion distribution for the reaction of acetic acid with NO^+ precursor ions at a water vapour concentration about 6% (typical for human breath), as can be seen in Fig. 3b.

3. Concluding remarks

One of the major problems in mass spectrometry involves the identification and quantification of isomeric and isobaric compounds. In this endeavour, gas chromatography mass spectrometry, GC-MS, has much to offer because of the additional compounds separation provided by the column and the varying elution times of compounds with different physical properties, even of structural isomers of the same general compound [29]. SIFT-MS is not so versatile, but the availability of three precursor ions, especially NO^+ in addition to H_3O^+, offers an opportunity to distinguish between some compounds of the same molecular weight by virtue of the different reaction processes that occur, a well documented case being the separation of aldehydes and ketones [8]. In the present paper it is shown how the two propanol isomers can be distinguished from the isobaric acetic acid and methyl formate on the basis of their reactions with H_3O^+ and NO^+ and, further, how the last two compounds can be distinguished from each other in humid samples using NO^+ precursor ions for SIFT-MS analysis. Thus, it is shown that acetic acid is present in mouth exhaled human breath at levels of typically 30–60 ppb in a few healthy individuals and lower by about a factor of two in nose exhaled breath. The principles involved in the present studies can be carried over to the detection and quantification of other compounds in complex media like exhaled breath, but the problems are compounded when they are present at trace levels. Nevertheless, the present study has shown that if a careful study of the ion chemistry occurring under the conditions of the individual SIFT-MS analytical instrument is made then even ostensibly intractable analyses can be performed.

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References

[1] S.S. Que Hee, Parent and progeny compounds in exhaled breath, Determination of, in: R.A. Meyers (Ed.), Encyclopedia of Analytical Chemistry, John Wiley & Sons, 2000.
[2] A. Amann, D. Smith, Breath Analysis for Clinical Diagnosis and Therapeutic Monitoring, World Scientific, Singapore, 2005.
[3] T.S. Wang, A. Pysanenko, K. Dryahina, P. Španěl, D. Smith, J. Breath Res. 2 (2008) 0370313.
[4] D. Smith, C. Turner, P. Španěl, J. Breath Res. 1 (2007) 014004.
[5] D. Smith, A.M. Diskin, Y. Ji, P. Španěl, Int. J. Mass Spectrom. 209 (2001) 81.
[6] P. Španěl, Y. Ji, D. Smith, Int. J. Mass Spectrom. Ion Process. 165/166 (1997) 25.
[7] D.A. Fairley, D.B. Milligan, C.G. Freeman, M.J. Mcewan, P. Španěl, D. Smith, Int. J. Mass Spectrom. 193 (1999) 35.
[8] D. Smith, P. Španěl, Mass Spectrom. Rev. 24 (2005) 661.
[9] K.P. Wyche, R.S. Blake, K.A. Willis, P.S. Monks, A.M. Ellis, Rapid Commun. Mass Spectrom. 19 (2005) 3356.
[10] P. Španěl, D. Smith, Int. J. Mass Spectrom. Ion Process. 172 (1998) 137.
[11] D. Smith, P. Španěl, Analyst 132 (2007) 390.
[12] D. Smith, T.S. Wang, P. Španěl, Physiol. Meas. 23 (2002) 477.
[13] P. Španěl, D. Smith, Int. J. Mass Spectrom. Ion Process. 167/168 (1997) 375.
[14] T.S. Wang, W. Carroll, W. Lenny, P. Boit, D. Smith, Rapid Commun. Mass Spectrom. 20 (2006) 125.
[15] P. Španěl, D. Smith, Rapid Commun. Mass Spectrom. 15 (2001) 563.
[16] D. Smith, T.S. Wang, P. Španěl, Int. J. Mass Spectrom. 230 (2003) 1.
[17] D. Smith, N.G. Adams, Adv. Atom. Mol. Phys. 24 (1987) 1.
[18] T. Su, W.J. Chesnavich, J. Chem. Phys. 76 (1982) 5183.
[19] D. Smith, A. Pysanenko, P. Španěl, Rapid Commun. Mass Spectrom. 23 (2009) 1419.
[20] P. Španěl, D. Smith, J. Am. Soc. Mass Spectrom. 12 (2001) 863.
[21] D. Smith, A. Pysanenko, P. Španěl, Int. J. Mass Spectrom. 281 (2009) 15.
[22] P. Španěl, K. Dryahina, D. Smith, Int. J. Mass Spectrom. 249/250 (2006) 230.
[23] P. Španěl, D. Smith, J. Breath Res. 2 (2008) 046003.
[24] P. Španěl, D. Smith, Rapid Commun. Mass Spectrom. 14 (2000) 1898.
[25] A.E. Jones, R.L. Summers, J. Emerg. Med. 19 (2000) 165.
[26] W. Vaughan, B. Gaston, T. MacDonald, E. Erwin, N. Malhotra, K. Zaman, T.A.E. Platts-Mills, J. Hunt, Eur. Respir. J. 18 (2001) 463.
[27] Z.F. Chen, B.W. Darvell, V.W.H. Leung, Arch. Oral. Biol. 49 (2004) 863.
[28] P. Martinez-Lozano, J.F. de la Mora, Anal. Chem. 80 (2008) 8210.
[29] J. Kubišta, P. Španěl, K. Dryahina, C. Workman, D. Smith, Rapid Commun. Mass Spectrom. 20 (2006) 563.