Analysis of Formaldehyde in the Headspace of Urine from Bladder and Prostate Cancer Patients Using Selected Ion Flow Tube Mass Spectrometry

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We have used selected ion flow tube mass spectrometry (SIFT-MS) to determine the concentration of formaldehyde in the headspace of urine from patients suffering from bladder and prostate cancer and from several healthy subjects as controls. We address the potential problems associated with the use of ion chemistry to quantify formaldehyde in the presence of the relatively large number densities of water molecules and show that formaldehyde can be quantified in urine headspace using analysis by SIFT-MS. These studies show that formaldehyde is clearly elevated in the headspace of the urine from the cancer patients as compared with urine from the healthy controls. Thus, with further improvements in the methodology and the sensitivity of our SIFT-MS technique, formaldehyde quantification in urine headspace using this new analytical method could be a valuable non-invasive indicator of the presence of early-stage tumours in the body. Copyright © 1999 John Wiley & Sons, Ltd.

We have developed a new analytical technique, selected ion flow tube mass spectrometry (SIFT-MS), for the real time detection and quantification of trace gases in air and breath.[1,2] The technical details of SIFT-MS have been described in detail in a previous paper[3] and a brief description is given in the next section. We have applied SIFT-MS to the analysis of trace gases in town air,[2] the volatile organic compounds emitted by food products[4] and especially to the analysis of human breath for clinical diagnosis and therapeutic monitoring.[1–3] Special studies have been made of ammonia in the breath of patients suffering from end-stage renal failure before and after haemodialysis[5,6] and the time variations of the concentrations of metabolites in the breath of healthy volunteers following a protein meal.[7] Most recently, we have developed a method for the analysis of ammonia, nitric oxide and volatile organic compounds emitted by urine at various pH[8] to explore another potential clinical application of this new analytical method.

Various studies have shown that certain breath metabolites may be indicators of the presence of tumours in the body, including the lungs,[9] and the presence of formalde-hyde on the breath of some patients suffering from breast cancer[10] has attracted some attention. It has also been shown that formaldehyde is emitted from tumour cells *in vitro*.[11] Stimulated by these studies, we have considered the use of SIFT-MS for the detection and quantification of formalde-hyde above urine and in breath as a potential diagnostic for the presence of tumours. However, there are potential problems when using SIFT-MS for formaldehyde analysis when relatively large number densities of water are also present as is the case for humid breath and the headspace above urine. In this paper we address these problems and show how analyses of formaldehyde in humid air can be achieved using SIFT-MS. We then present the results of a preliminary study of formaldehyde present in the headspace of urine from patients suffering from bladder and prostate cancer. We make no attempt here to consider the detailed clinical implications of the results of this study; these will be discussed in a subsequent paper.

EXPERIMENTAL

The SIFT/MS analytical technique

A prototype SIFT mass spectrometer located at Keele University (custom-made by Europa Scientific Ltd., Crewe, UK, according to the design by Smith and Španěl,[1] was used to quantify the concentration of formaldehyde in urine headspace. The SIFT-MS technique utilises chemical ionisation in a flow tube.[1–3] Precursor ions of a given mass-to-charge ratio selected by a quadrupole mass filter are injected into fast flowing helium carrier gas where they

thermalise to the carrier gas temperature (300 K) and then react with the trace gases in a sample of air, breath or urine headspace which is introduced into the carrier gas downstream. The precursor ions of choice are H_3O^+, NO^+ and O_2^+ because these do not react with the major constituents of air and breath (N_2, O_2, CO_2, H_2O, Ar) but variously react with most species that appear as trace gases in polluted air, breath and above liquids thus producing characteristic product ions. A downstream quadrupole mass spectrometer is scanned and detects and counts the precursor and product ions of the reactions as the air, breath or headspace sample flows into the helium carrier gas. Thus, the mass spectrum over a predetermined mass range is recorded by an on-line computer and the several trace gases in the sample can be identified and their concentrations immediately determined.[3,6,8] This describes SIFT-MS operated in the full scan mode as is exclusively used in the present experiments.

To analyse air (or breath) samples in which the concentrations of trace gases vary with time, the selected ion monitoring mode[2,3] can be used in which the downstream mass spectrometer is switched rapidly between the selected precursor ion and several chosen product ions and their count rates are stored and analysed by the on-line computer. In this way, variations in trace gas concentrations can be monitored in real time, including the rapid variations that occur in single breath exhalations[2,3] and the much slower variations in the emissions of volatile organic compounds from some foods.[4]

These analyses by SIFT-MS provide absolute concentrations of trace gases present in air or breath sampled directly at constant (usually atmospheric) pressure and a known flow rate, and the measured concentrations are usually expressed in parts per billion (ppb) or parts per million (ppm). However, when sampling from liquid headspace from a fixed volume the sample pressure may reduce with time and then the derived (varying) concentrations of trace gases have only relative significance. Further details of the operation of the SIFT-MS technique are available elsewhere.[1,2,3,8]

Essential to these analyses by SIFT-MS is the kinetics database of the reactions of H_3O^+, O_2^+ and NO^+ which we have constructed from detailed studies of the reactions of these ions with a wide variety of organic (and some inorganic) species.[12–18] H_3O^+ ions can be used to detect and quantify most organic vapours and ammonia; they transfer their protons to the different trace gases, M, in the sample producing MH^+ ions (e.g. H_3CO^+ for formaldehyde), and it turns out that only H_3O^+ (and not NO^+ and O_2^+) is suitable for formaldehyde analysis. (We have used O_2^+ ions to detect nitric oxide in urine headspace.[8]) Since urine headspace is very humid, a fraction of the product MH^+ ions react with the abundant water molecules to form the monohydrate and sometimes the dihydrate ions, $MH^+ \cdot H_2O$ and $MH^+ \cdot (H_2O)_2$, and these must be included in the precursor and product ion count rates for accurate quantification of individual trace gases.[3] However, the situation for formaldehyde analysis is not so straightforward as we will see.

Urine samples

In these preliminary studies, urine samples were collected from 14 patients with bladder cancer, 24 patients with prostate cancer and from 14 healthy volunteers. Most of the samples were immediately frozen and stored at a tempera-

ture of $-70\,°C$ to await analysis. Tests showed that there was no apparent deterioration of these urine samples even when stored this way for some weeks. It is known that vapour release from urine will sometimes be dependent on its pH, but this is not the case for many volatile organic compounds such as ethanol and acetone.[8] Nevertheless, we considered it important to investigate if pH is important for formaldehyde release from urine. Normal urine is slightly acidic at a pH of about 6.7. In these experiments, 10 mL portions of urine were introduced into glass bottles of volume approximately 200 mL closed with a septum and, by the addition of appropriate amounts of 0.1 M HCl and 0.1 M NaOH, urine samples at pH values of 4 and 8 (in addition to samples at their unchanged pH) were prepared. The bottles with the samples were then placed in a thermostatically controlled oven to raise their temperature to 40 °C. The headspace above each urine sample was then sampled by puncturing the septum with a needle connected directly to the inlet port of the SIFT mass spectrometer. The mass spectra were then acquired using H_3O^+ precursor ions over 50 s integration periods.

RELEVANT ION CHEMISTRY

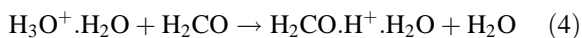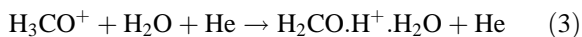The protonation of formaldehyde by H_3O^+ proceeds by the reaction:

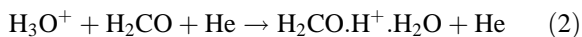$$H_3O^+ + H_2CO \rightarrow H_3CO^+ + H_2O \qquad (1)$$
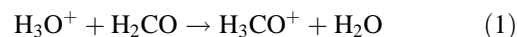
This proton transfer reaction is exothermic because the proton affinity of formaldehyde, $PA(H_2CO)$, which is 718 kJ mol^{-1},[19] exceeds that of water, $PA(H_2O)$, which is 697 kJ mol^{-1}.[19] Clearly, this PA difference is not great and so at a reaction temperature of 300 K, at which the present SIFT-MS experiments were carried out, the reverse reaction may occur, resulting in a loss of the product H_3CO^+ ions. The production rate of H_3CO^+ ions by reaction (1) is given by $\kappa_1[H_3O^+][H_2CO]$, where κ_1 is the rate coefficient of the forward reaction and the square brackets refer to the number densities of the enclosed species in the helium carrier gas. Similarly, the loss rate of H_3CO^+ by the reverse reaction (1) is given by $\kappa_1[H_3CO^+][H_2O]$. κ_1 has been measured to be 3×10^{-9} cm^{3}s^{-1} at 300 K[20] indicating that the reaction occurs at the collisional rate.[21] An early SIFT measurement of κ_1 indicated a value of about 3×10^{-11} cm^{3}s^{-1} at 300 K,[22] which in view of the known $PA(H_2CO)$ must be too large for a proton transfer reaction that is 21 kJ mol^{-1} endothermic. A subsequent measurement indicated a value for κ_1 of about 5×10^{-13} cm^{3}s^{-1}.[23] This is consistent with an estimated upper limit value of 10^{-12} cm^{3}s^{-1} obtained using the simple relationship $\kappa_1 = \kappa_{1e}^{-E/RT}$, where E is the endothermicity of the reaction (which neglects any small entropy change in reaction (1)). It follows from these known kinetics that the time constant, τ, for the loss of H_3CO^+ ions by the backward reaction (1) ($\tau = (k_{-1}[H_2O])^{-1}$), even at the relatively large number water molecule densities of up to 10^{13} cm^{-3} which inevitably result when humid air or urine headspace gas is introduced into the helium carrier gas, is very long (about 200 ms) relative to the reaction time (typically 5 ms[2,3]). Thus at 300 K the loss of H_3CO^+ ions by this reaction and the corresponding error in the determination of the H_2CO number density are insignificant.

However, it is apparent in these experiments that when formaldehyde is present in the flow tube and H_3O^+ is the precursor ion there is a low level ion signal in the SIFT mass

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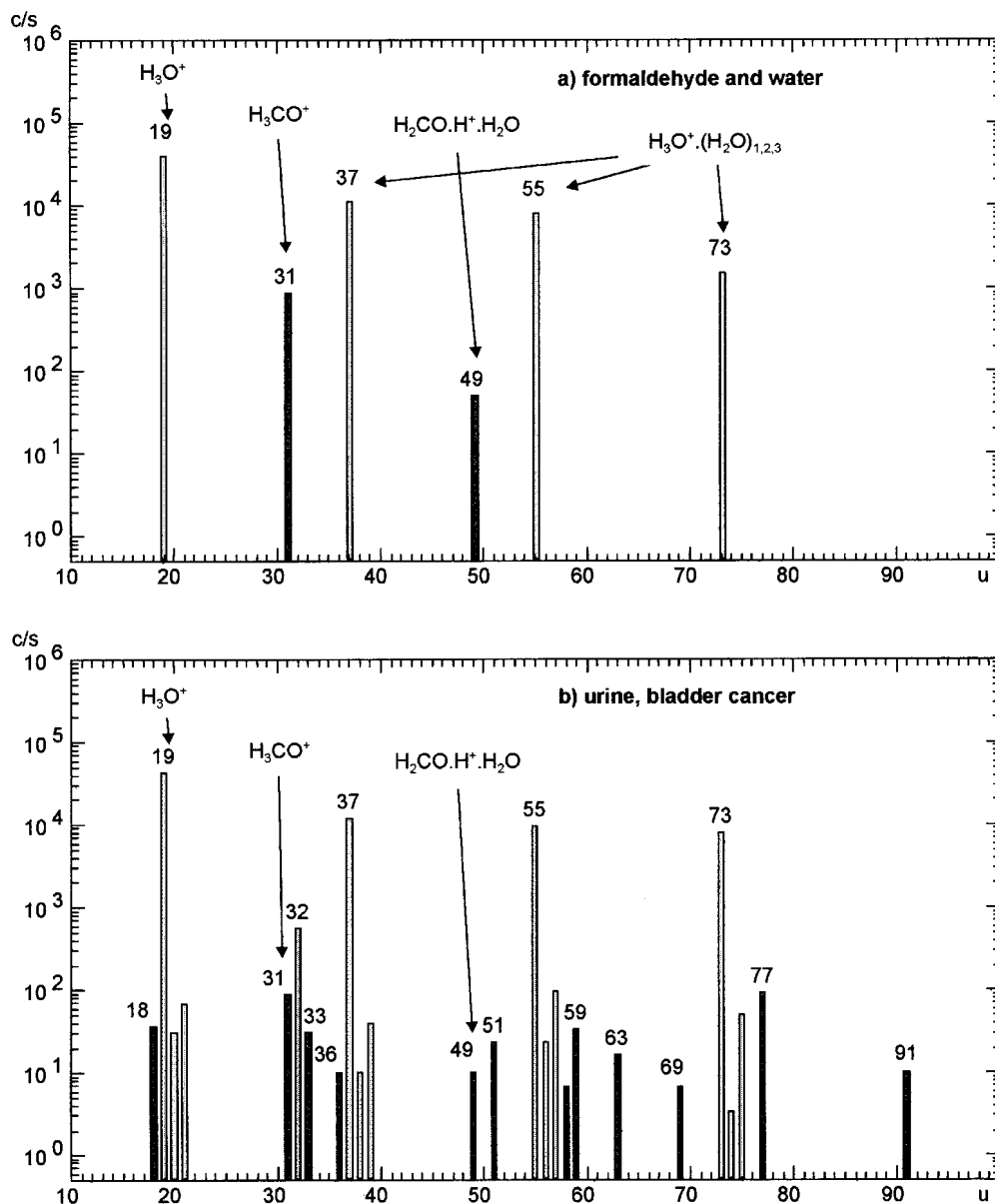
Figure 1. Sample SIFT mass spectra: (a) formaldehyde and water vapour introduced into the helium carrier gas; the open bars are the precursor hydronium ions (19 u) and the product hydrated hydronium ions (37, 55, 73 u); the solid bars are protonated formaldehyde and its monohydrate (31 and 49 u). (b) urine headspace from a bladder cancer patient; note the presence of the formaldehyde-derived ions (31, 49 u) and ions derived from ammonia (18, 36 u), methanol (33, 51, 69 u) and acetone (59, 77 u).

spectra at mass 49 u, as can be seen in the sample spectra shown in Fig. 1. This is surely the proton-bound ion $H_2CO.H^+.H_2O$. There are three reactions that can form this ion:

$$H_3O^+ + H_2CO + He \rightarrow H_2CO.H^+.H_2O + He \quad (2)$$

$$H_3CO^+ + H_2O + He \rightarrow H_2CO.H^+.H_2O + He \quad (3)$$

$$H_3O^+.H_2O + H_2CO \rightarrow H_2CO.H^+.H_2O + H_2O \quad (4)$$

The first two are three-body (termolecular) association reactions in which collisions of the excited intermediate adduct ions $(H_2CO.H^+.H_2O)^*$ with helium carrier gas atoms result in their stabilisation against dissociation back to the reactants. It is unlikely that reaction (2) can compete with the rapid exothermic bimolecular forward reaction (1),

whereas reaction (3) can compete with the much slower (endothermic) reverse of reaction (1). Then reaction (3) would represent a loss of the H_3CO^+ ions from the flow tube which would distort the analysis of the H_2CO concentration if not accounted for. At first glance, a simple way to account for this loss would be to include the count rate of mass 49 u together with the majority 31 u in the quantification of formaldehyde. However, a complication arises in the form of reaction (4) and its reverse which represent formation and loss of the 49 u ion. It has been shown previously by studies of H_2CO/H_2O ion chemistry[23] that the equilibrium constant for reaction (4) is 5.7 at 300 K and that the rate coefficient of the reverse reaction (4) is 5×10^{-10} cm^{3}s^{-1}. (this reaction is apparently slightly endothermic[24]). Although the rate coefficient for the forward exothermic switching reaction (4) is expected to exceed that of its endothermic reverse
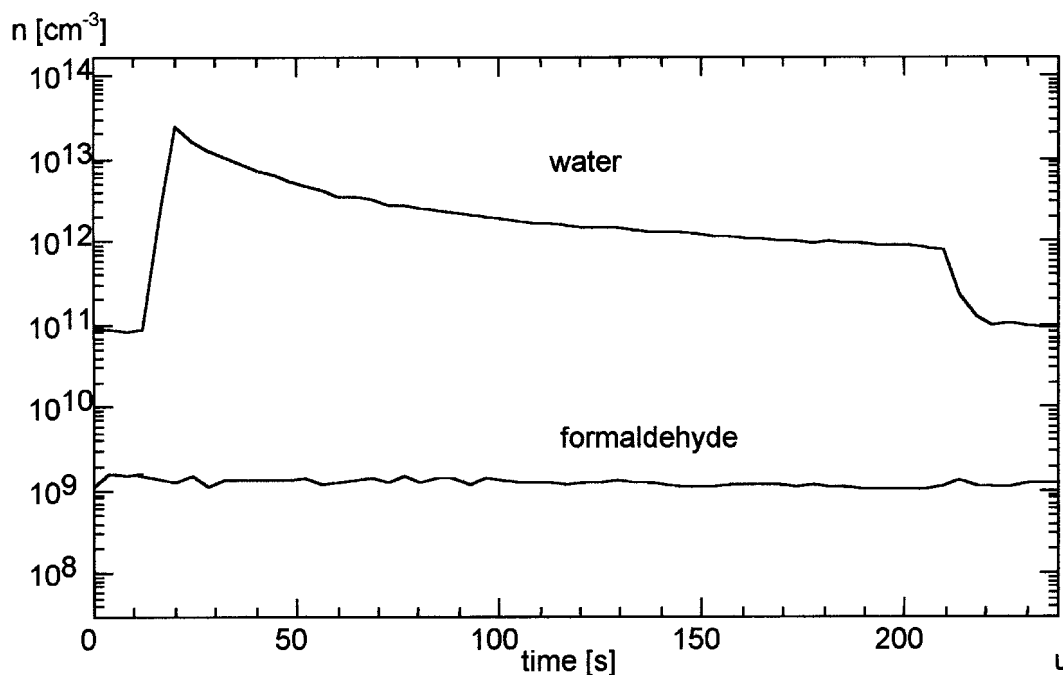
Figure 2. Determinations of the number densities, n, of water and formaldehyde molecules in the flow tube during SIFT/MS. The formaldehyde flowed into the carrier gas at a constant rate whereas the water vapour flow rate was allowed to reduce over the wide range indicated over a period of about 200 s. Note that the formaldehyde number density as determined using the $[H_3CO^+]/[H_3O^+]$ ratio of ions signals is independent of the water vapour number density.

reaction, since $[H_2O]$ greatly exceeds $[H_2CO]$ in the present experiments, the net result is that the reverse reaction (4) will significantly reduce the $[H_2CO.H^+.H_2O]$ ions in the flow tube. This largely explains why the 49 u ion is always at a very small count rate relative to the 31 u ion (see Fig. 1). So the simple inclusion of the mass 49 u ion together with the 31 u ion in the quantification of the formaldehyde is not valid in this case and it is necessary to consider the influence of reaction (3) on the loss of the H_3CO^+ ions.

Our many observations on the reactions of H_3O^+ and H_3CO^+ ions in SIFT experiments have indicated that the termolecular rate coefficient for reaction (3) ($k_3 = 7 \times 10^{-28}$ cm^{6}s^{-1} at 300 K[20]) is closely equal to that for the termolecular reaction:

$$H_3O^+ + H_2O + He \rightarrow H_3O^+.H_2O + He \qquad (5)$$
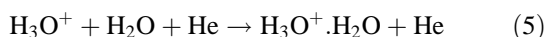
which is the major loss process for H_3O^+ in SIFT-MS experiments. These observations are thus consistent with the recent measurement of k_3 of 5.7×10^{-28} cm^{6}s^{-1}[24] obtained in a selected ion flow drift tube experiment at a mean interaction energy of 0.05 eV which is only slightly greater than that appropriate to these SIFT-MS experiments (i.e. 0.038 eV) which were conducted at truly thermal energies at 300 K. So the rates of loss of both H_3CO^+ and H_3O^+ ions in termolecular reactions with water are comparable. Thus it should be valid to consider the ratio of the ion signals $[H_3CO^+]/[H_3O^+]$ for the quantification of formaldehyde in the flow tube.

However, in view of the relative complexity of this ion chemistry, we considered it necessary to experimentally demonstrate that the water concentration in the flow tube does not seriously influence the determination of the

formaldehyde concentration from the $[H_3CO^+]/[H_3O^+]$ signal ratio. Thus, a constant flow of formaldehyde was introduced into the flow tube and then water vapour was also introduced initially at a relatively high concentration which was allowed to reduce by about two orders of magnitude over a period of about 200 s during which time the formaldehyde number density (determined from the $[H_3CO^+]/[H_3O^+]$ ratio only) was obtained. The data obtained are shown in Fig. 2. They graphically illustrate that the formaldehyde concentration obtained this way is not significantly influenced by the presence of the water over the wide number density range from 10^{11} cm^{-3} to in excess of 10^{13} cm^{-3} which covers the $[H_2O]$ levels expected in all our analyses by SIFT-MS of urine headspace and breath.

RESULTS AND DISCUSSION

Influence of pH on formaldehyde headspace concentration

We first considered the influence of the pH of the urine on the release of formaldehyde. Thus, the mean formaldehyde headspace concentrations were treated statistically for the 156 samples of the acidic (pH = 4), the normal pH (typically 6.7) and the alkaline (pH = 8) urine obtained from 52 subjects (38 patients, 14 controls). No significant differences in the headspace formaldehyde concentrations were observed with pH in common with the headspace concentrations of the other volatile organics emitted by the urine[8] which were also obtained using SIFT-MS in the full scan mode. Thus, the data presented below are the mean concentrations of the triplicate formaldehyde determinations in the headspace of the urine from each patient and each control.
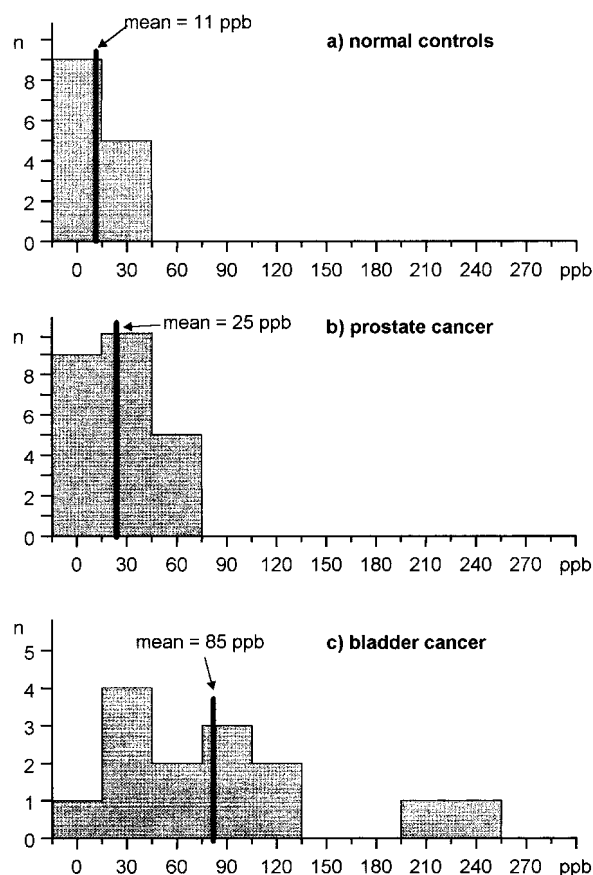
Figure 3. Histograms of the distributions of formaldehyde concentrations (in ppb) found in the headspace of urine for the three groups indicated. The concentrations are grouped over 30 ppb intervals.

Results of the analyses

The results presented in Fig. 3 are in the form of separate histogram distributions of urine headspace formaldehyde for the 14 controls, the 24 prostate cancer patients and the 14 bladder cancer patients. It is immediately obvious by inspection that the formaldehyde levels are greatest in the headspace of the urine from the bladder cancer patients and the smallest in the headspace of the healthy controls. Indeed, no formaldehyde was detected (detection limit 10 ppb) in the urine headspace from the majority of the controls, whereas it was detected in the headspace of the urine from all but one of the 14 bladder cancer patients. The urine from the prostate cancer patients had formaldehyde concentrations intermediate between those of the bladder cancer patients and the controls. A simple statistical analysis of variance (ANOVA) of these data clearly indicates significant differences in the formaldehyde concentrations from the three groups ($p = 0.0005$ for the difference between the controls and the bladder cancer patients and $p = 0.05$ for the difference between the controls and the prostate cancer patients). To emphasise this point, the mean values of formaldehyde concentrations (in ppb) are given in Fig. 3 for each group. We only note here that the tumours in both the prostate and bladder cancer patients were at various clinically-defined stages and that some were in remission; this must surely influence the formaldehyde concentrations in the urine. A more thorough statistical analysis of these data will be presented in a later paper when their clinical significance will be properly assessed and reported.

CONCLUDING REMARKS

It is clear from these preliminary SIFT-MS studies that formaldehyde is elevated in the headspace of the urine from patients suffering from bladder and prostate cancer. Further, it is clear that the formaldehyde is at a greater concentration in the urine from the bladder cancer patients than in the urine from prostate cancer patients. This is perhaps not surprising in that bladder tumours are in closer contact with the urine whereas the formaldehyde from prostate tumours must generally progress into the urine via the blood stream. However, these clinical differences must be more thoroughly assessed and established by further consideration of the present data and by the analyses of many more urine samples using SIFT-MS in carefully controlled clinical trials.

We are now working to improve the intrinsic detection sensitivity of our SIFT-MS analytical method towards the 1 ppb level (from the current 10 ppb level). Further improvements in the urine headspace sampling methodology can surely be realised also (e.g. larger sample volume; higher sample temperature). When these are achieved, we hope to be able to detect the presence of tumours at the preclinical stage with all the benefits that this would bring. We have also carried out preliminary formaldehyde analyses of the breath of some patients with lung cancer with encouraging results. It seems that it may be possible in the future to detect the presence of body tumours non-invasively from a breath and/or a urine test using further developed SIFT-MS technology.

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