Analysis of volatile emissions from porcine faeces and urine using selected ion flow tube mass spectrometry

D. Smith a,*, P. Španěl b, J.B. Jones c

a Department of Medical Physics, School of Postgraduate Medicine, Keele University, Thornburrow Drive, Hartshill, Stoke-on-Trent, Staffordshire ST4 7QB, UK
b J. Heyrovsky Institute of Physical Chemistry, Academy of Sciences of the Czech Republic, Dolejskova 3, Prague 8 182 32, Czech Republic
c Silsoe Research Institute, Wrest Park, Silsoe, Bedfordshire MK45 4HS, UK

Received 20 November 1999; received in revised form 12 February 2000; accepted 15 February 2000

Abstract

The headspaces above faecal and urine samples provided by six pigs have been analysed using selected ion flow tube mass spectrometry (SIFT/MS). The concentrations of ammonia, nitric oxide, nitrogen dioxide, acetone, methanol, ethanol, dimethyl sulphide (DMS) and low molecular weight carboxylic acids in the headspace volume were measured down to the parts per billion (ppb) levels. The differences in the relative concentrations of these compounds between faeces and urine, the implications of these differences and the advantages of using SIFT/MS in such diagnostic and physiological monitoring work are discussed. © 2000 Elsevier Science Ltd. All rights reserved.

Keywords: Selected ion flow tube mass spectrometry; Pigs; Urine; Faeces; Animal physiology

1. Introduction

An obstacle to identifying and quantifying the major compounds present in released vapours from the breakdown of animal excreta, including urine and faeces, is the absence of a rapid, comprehensive, reproducible, sensitive analytical method. At present, gas chromatography is the preferred analytical method (e.g., Elliott et al., 1978; Hobbs et al., 1995). Though reliable and reproducible, gas chromatography is selective and slow, and often requires the samples to be concentrated before analysis (e.g., Phillips and Greenberg, 1992). Direct input mass spectrometry is commonly used alongside gas chromatograph to speed up analysis. Though the low-pressure electron impact ion source of conventional mass spectrometers allows identification of the volatile compounds present in complex mixtures, it usually does not allow accurate quantitative analyses (Španěl and Smith, 1996). Other available methods such as photoionisation detectors and electronic noses, which are rapid and sensitive and do not require the pre-concentration of samples, are still range limited. For example, photoionisation can detect only compounds whose ionisation energies are less than the energy of the photons emitted by the source, while electronic noses need to have their chemical sensors tailored to certain groups of compounds in order to optimise discrimination.

In contrast, selected ion flow tube mass spectrometry (SIFT/MS) is a rapid, sensitive and reproducible technique for quantitative analysis of trace gases in air (Smith and Španěl, 1996a,b; Španěl and Smith, 1996). SIFT/MS exploits chemical ionisation rather than electron impact ionisation. Because the former minimises fragmentation of organic molecules before they are detected, the mass spectra have fewer ion peaks. Identification of molecules is therefore easier and quantification more accurate. In the present study, we illustrate these advantages of SIFT/MS by employing this new analytical method to quantify the major compounds that are released into the headspace above urine and faeces excreted by pigs.

2. Methods

2.1. Pigs and husbandry

Six female Duroc × Landrace cross-bred pigs (Sus scrofa) were weaned from a commercial outdoor...
breeding herd at three–four weeks of age. The pigs were then housed indoors in holding pens bedded with wheat straw. The holding pens were lit on a 16:8 h light/dark cycle and food and water was provided ad libitum. The pigs were exclusively fed a weaner diet (primary select, primary diets, Ripon, UK).

2.2. Collection and storage of urine and faecal samples

The urine and faeces were collected from pigs held in enclosed crates at Silsoe. The metal crates measured 1.32 m × 0.55 m × 0.79 m (length × width × height) and were raised 0.82 m above the floor. The fronts and backs of the crates were formed from removable metal panels, which facilitated entry to the crates. One side of each crate was formed from a solid/weld-mesh panel and the other side from metal bars. Weld-mesh covered the floor and roof of the crates. The faeces collected on the weld-mesh floor. A metal funnel located beneath a 0.66 m × 0.5 m area of the floor allowed the urine to drain into a bucket. A metal ramp, inclined at an angle of 11°, was positioned over the area of the floor not covered by the funnel to direct faeces and urine into the collection area. Water was available from a bowl fixed to the top of this ramp.

Once excreted, the faeces and urine samples were collected in separate 240 ml glass amber septum bottles with Teflon/silicone septa hole screw caps (Supelco, Poole, UK) and initially stored at approximately 4°C. All the samples were frozen at approximately −20°C within four hours of being collected and transported to Keele University for SIFT–MS analysis. The reason for freezing the samples was to inhibit biochemical and bacterial processes prior to analysis. Clearly, the vapour emissions from these freshly collected samples do not represent vapour emissions from maturing slurry (Hobbs et al., 1995). Any effects that this freezing procedure might have will be investigated experimentally when SIFT–MS analyses are immediately possible following the excretion. Meanwhile, it is worthy of note that vapour emissions from human urine samples as analysed using SIFT–MS are not significantly changed by freezing (Smith et al., 1999a). Presumably, any changes would be similar for all the samples within this study and would not greatly influence the relative values of the detected concentrations.

2.3. Preparation of samples for SIFT/MS analysis

The urine and faecal samples were thawed to room temperature and the faeces mixed with de-ionised water to form a solution (weight (g):volume (ml) ratio of 1:1). It is well understood that vapour release from liquids will generally be dependent on the pH of the liquid and this is certainly so for human urine (Smith et al., 1999a). Hence, three 10-ml aliquots of each urine and faecal sample were placed in separate 240-ml glass amber septum bottles. The pH of one aliquot was unchanged. The pH of another aliquot was reduced to 4.0 using 0.1M hydrochloric acid and the pH of the third aliquot increased to 8.0 using 0.1M sodium hydroxide. Each aliquot was sealed with Teflon/silicone septa hole screw cap then stored at approximately 2.5°C. All aliquots were analysed by SIFT/MS within 24 h.

2.4. SIFT/MS analysis

The SIFT/MS technique utilises chemical ionisation in a flow tube (Smith and Španel, 1996a,b; Španel and Smith, 1996). Precursor ions of a given mass-to-charge ratio (selected by a quadrupole mass filter) are injected into fast flowing helium carrier gas. There they react during a defined reaction time with the trace gases in a sample of air, breath or liquid headspace, which is introduced into the carrier gas at a known flow rate, and produces characteristic product ions. A downstream quadrupole mass spectrometer is used to detect and count the precursor and product ions. The resultant mass spectrum (over a pre-determined mass range) is fed into an online computer which immediately identifies and quantifies the trace gases in the sample (Smith and Španel, 1996a,b; Španel and Smith, 1996). H2O+ precursor ions are 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., CH3COCH3·H+, protonated acetone; NH4+, protonated ammonia). Since liquid headspace is usually very humid, a fraction of the product MH+ ions reacts with the water molecules to form the monohydrate, MH+(H2O), and sometimes the dihydrate, MH+(H2O)2. These hydrated ions are included in the product ion count for accurate quantification of the individual trace gases in the headspace gas (Španel and Smith, 1996). O2 ions can be used to confirm these analyses and additionally to detect certain inorganic species, including NO and NO2, which they ionise producing M+ ions (e.g., NO+, ionised nitric oxide). Essential for this SIFT/MS analytical method is the kinetics database of the reactions of H2O+ and O2 constructed from detailed studies of the reactions of these ions with a wide variety of compounds (Španel and Smith, 1997; Španel and Smith, 1999).

The bottles containing the urine and faecal samples (at the three different pH values) were 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/MS. Mass spectra were then recorded using H3O+ precursor ions to detect and analyse the polar molecules.
(e.g., ammonia, acetone, methanol, ethanol, dimethyl sulphide (DMS) and carboxylic acids) and using $O_2^+$ precursor ions to detect nitric oxide and nitrogen dioxide (Smith et al., 1999a). Each mass spectrum was acquired for 50 s in these analyses.

Provided the pressure of the headspace remains substantially unchanged (i.e., a large volume) during the sampling period, measured concentrations can be accurately expressed in parts per billion (ppb) or parts per million (ppm). When the headspace is of a limited volume (as in the present studies) the headspace pressure reduces with time. Therefore the derived concentrations of various components have only relative significance. The mean water vapour concentration in the headspace is routinely measured in these analyses. Small variations of the water vapour concentration between the samples were evident due to small variations in the temperatures of the sample liquids. To account for this, the derived concentrations of the trace gases were normalised to the greatest water vapour concentration within each set of the three urine and faecal samples.

3. Results and discussion

A total of thirty-six SIFT/MS spectra were obtained for both the faecal and urine samples (faeces/urine samples from six pigs at three pH levels using two precursor ions). Fig. 1(a) and (b) shows typical sample spectra obtained from the headspace of an acidified faeces/water sample at pH 4 using $H_3O^+$ precursor ions $(m/z = 19$) in the presence of their hydrates $H_3O^+(H_2O)_{1,3}$ $(m/z = 37; 55$ and $73$). Reactions of these ions with the volatile compounds in the sample form the product ions indicated (ammonia, $Am$; methanol, $M$; ethanol, $E$; acetone, $A$; dimethyl sulphide, DMS; acetic acid, Ac; propanoic acid Pr; butanoic acid, Bu; pentanoic acid, Pe). (b) Obtained using $O_2^+$ precursor ions $(m/z = 32)$. NO, NO$_2$ and DMS are also detected as indicated.

![Fig. 1. Typical SIFT/MS spectra obtained for the headspace above the samples of faeces in acidic solution at pH 4. Note that all the mass spectra in this paper are represented on a semi-logarithmic scale using counts per second as a measure of the detected ion signal levels. (a) Obtained using $H_3O^+$ precursor ions $(m/z = 19)$ in the presence of their hydrates $H_3O^+(H_2O)_{1,3}$ $(m/z = 37; 55$ and $73)$. Reactions of these ions with the volatile compounds in the sample form the product ions indicated (ammonia, $Am$; methanol, $M$; ethanol, $E$; acetone, $A$; dimethyl sulphide, DMS; acetic acid, Ac; propanoic acid Pr; butanoic acid, Bu; pentanoic acid, Pe). (b) Obtained using $O_2^+$ precursor ions $(m/z = 32)$. NO, NO$_2$ and DMS are also detected as indicated.](image-url)
Typical sample spectra are presented in Fig. 2(a) and (b), respectively for acidified urine headspace analysed by $\text{O}_2^-$ ions and alkaline urine (pH 8.0) analysed using $\text{H}_3\text{O}^+$ ions. Again, in the acidified urine headspace, NO and NO$_2$ are both evident while very high concentrations of ammonia are present above the alkaline urine.

### 3.1. Faecal samples

Table 1 lists the concentrations in ppb of the major compounds released into the headspace above the faecal samples from each of the six pigs. The ammonia concentrations are those recorded above the alkaline samples, whilst the concentrations of carboxylic acids, NO and NO$_2$ are those recorded above the acidic samples. The methanol, ethanol, acetone and DMS concentrations that are given are the mean values of their concentrations above the acidic, normal pH and alkaline samples. Previous SIFT/MS analyses of the headspace of human urine (Smith et al., 1999a) have shown that the headspace concentrations of these organic molecules do not vary greatly with the pH of the urine and this was confirmed by the present studies. Hence the reason for reporting the mean values of the concentrations of these organic compounds.

The ammonia concentration varied greatly amongst the faecal samples from the six pigs, with that from Pig A sample being especially low and the sample from Pig B being exceptionally high (see Table 1). The presence of ammonia in the faeces may be the result of bacterial action (Lunberg et al., 1997; Smith et al., 1999a) (see also below). A wide variation in the concentrations of the organic molecules is not apparent, although the acetone level above the faeces from Pig A (see Table 1) was very low on all the three samples at the different pH values. It is interesting to note that in human physiology, we consistently see breath acetone levels maximise during fasting and minimise rapidly following feeding with a protein meal when breath ammonia levels increase towards maximum levels (Smith et al., 1999b). That both the ammonia and acetone levels are relatively small in the faeces of Pig A is not consistent with this.
Table 1
Concentrations of volatile compounds (ppb) present in the headspace above the faecal samples

<table>
<thead>
<tr>
<th>Pig</th>
<th>Ammonia</th>
<th>Nitric oxide</th>
<th>Nitrogen dioxide</th>
<th>Acetone</th>
<th>Methanol</th>
<th>Ethanol</th>
<th>DMS</th>
<th>Acetic acid</th>
<th>Propanoic acid</th>
<th>Butanoic acid</th>
<th>Pentanoic acid</th>
<th>Hexanoic acid</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>4123</td>
<td>663</td>
<td>158</td>
<td>302</td>
<td>2337</td>
<td>8214</td>
<td>2068</td>
<td>5391</td>
<td>5136</td>
<td>2777</td>
<td>591</td>
<td>86</td>
</tr>
<tr>
<td>B</td>
<td>11355</td>
<td>4077</td>
<td>1989</td>
<td>4952</td>
<td>1764</td>
<td>8968</td>
<td>4859</td>
<td>3080</td>
<td>1539</td>
<td>1409</td>
<td>327</td>
<td>65</td>
</tr>
<tr>
<td>C</td>
<td>43160</td>
<td>359</td>
<td>637</td>
<td>2052</td>
<td>1102</td>
<td>5671</td>
<td>2052</td>
<td>1738</td>
<td>1521</td>
<td>923</td>
<td>272</td>
<td>55</td>
</tr>
<tr>
<td>D</td>
<td>10854</td>
<td>897</td>
<td>128</td>
<td>7455</td>
<td>513</td>
<td>8132</td>
<td>2045</td>
<td>1782</td>
<td>1948</td>
<td>919</td>
<td>390</td>
<td>111</td>
</tr>
<tr>
<td>E</td>
<td>5895</td>
<td>536</td>
<td>593</td>
<td>3347</td>
<td>1314</td>
<td>6985</td>
<td>2148</td>
<td>3080</td>
<td>1822</td>
<td>441</td>
<td>252</td>
<td></td>
</tr>
<tr>
<td>F</td>
<td>6469</td>
<td>1328</td>
<td>245</td>
<td>3351</td>
<td>448</td>
<td>6977</td>
<td>970</td>
<td>2253</td>
<td>2374</td>
<td>564</td>
<td>362</td>
<td>81</td>
</tr>
<tr>
<td>Mean</td>
<td>30675</td>
<td>1310</td>
<td>625</td>
<td>3577</td>
<td>1246</td>
<td>7491</td>
<td>2357</td>
<td>2887</td>
<td>2621</td>
<td>1402</td>
<td>397</td>
<td>108</td>
</tr>
<tr>
<td>S.D.</td>
<td>43179</td>
<td>1396</td>
<td>703</td>
<td>2453</td>
<td>729</td>
<td>1179</td>
<td>1304</td>
<td>1363</td>
<td>1383</td>
<td>804</td>
<td>111</td>
<td>73</td>
</tr>
</tbody>
</table>

The concentrations have been normalised against the faecal sample with the greatest water concentration in each three samples at the three different pH values. Also given are the mean values and the S.D. for each compound.

Table 2
Concentrations of volatile compounds (ppb) present in the headspace above the urine samples

<table>
<thead>
<tr>
<th>Pig</th>
<th>Ammonia</th>
<th>Nitric oxide</th>
<th>Nitrogen dioxide</th>
<th>Acetone</th>
<th>Methanol</th>
<th>Ethanol</th>
<th>DMS</th>
<th>Acetic acid</th>
<th>Propanoic acid</th>
<th>Butanoic acid</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>47753</td>
<td>237</td>
<td>0</td>
<td>190</td>
<td>178</td>
<td>379</td>
<td>91</td>
<td>94</td>
<td>362</td>
<td>65</td>
</tr>
<tr>
<td>B</td>
<td>99887</td>
<td>1808</td>
<td>450</td>
<td>635</td>
<td>357</td>
<td>886</td>
<td>254</td>
<td>1594</td>
<td>1107</td>
<td>320</td>
</tr>
<tr>
<td>C</td>
<td>36871</td>
<td>387</td>
<td>230</td>
<td>201</td>
<td>101</td>
<td>290</td>
<td>146</td>
<td>1240</td>
<td>798</td>
<td>221</td>
</tr>
<tr>
<td>D</td>
<td>49241</td>
<td>1066</td>
<td>102</td>
<td>162</td>
<td>99</td>
<td>152</td>
<td>109</td>
<td>260</td>
<td>476</td>
<td>0</td>
</tr>
<tr>
<td>E</td>
<td>48520</td>
<td>1713</td>
<td>0</td>
<td>656</td>
<td>216</td>
<td>367</td>
<td>99</td>
<td>565</td>
<td>292</td>
<td>27</td>
</tr>
<tr>
<td>F</td>
<td>21075</td>
<td>173</td>
<td>0</td>
<td>130</td>
<td>37</td>
<td>183</td>
<td>40</td>
<td>212</td>
<td>429</td>
<td>0</td>
</tr>
<tr>
<td>Mean</td>
<td>50558</td>
<td>897</td>
<td>130</td>
<td>329</td>
<td>165</td>
<td>376</td>
<td>123</td>
<td>661</td>
<td>577</td>
<td>106</td>
</tr>
<tr>
<td>S.D.</td>
<td>26471</td>
<td>741</td>
<td>181</td>
<td>246</td>
<td>114</td>
<td>266</td>
<td>73</td>
<td>616</td>
<td>313</td>
<td>134</td>
</tr>
</tbody>
</table>

The concentrations have been normalised against the urine sample with the greatest water concentration in each three samples at the three different pH values. Also given are the mean values and the S.D. for each compound.
human breath scenario. The acetone, methanol, ethanol and DMS levels vary over relatively narrow limits. These organic compounds result from bacterial action in the gut (Prins, 1977) and so it is not a surprise to record their presence in the faeces. This is also the case for the fatty acids, which were most concentrated above the acidified faeces, their concentrations decreasing along the homologous series, with acetic acid being at the highest concentration. This does not necessarily reflect the relative concentrations of these acids in the liquid phase, because the partition of the acids between the liquid and vapour phases will favour the low molecular weight species due to their larger saturated vapour pressures.

The presence of both NO and NO₂ in the acidified faecal/water sample headspace is indicative of aerobic bacterial action. Their presence above acidified human urine is an indicator of urinary tract bacterial infection, which has been confirmed by culture tests (Smith et al., 1999a). The bacteria reduce the nitrates that are naturally present in urine to nitrites (Lunberg et al., 1997), which are converted to nitrous acid, HONO₂, when the solution is acidified. The HNO₂ in turn partially dissociates to NO and NO₂ (and water). Thus, NO, NO₂ and HNO₂ flow into the SIFT/MS where they are detected and quantified by O₂⁻ ions, the NO resulting in NO⁺ ions, the NO₂ resulting in NO₂⁻ ions and the HNO₂ resulting in both NO⁺ and NO₂⁻ ions.

### 3.2. Urine samples

Table 2 lists the concentrations in ppb of the major compounds released into the headspace above the urine samples from each of the six pigs. Again, the ammonia concentrations were recorded above the alkaline samples, the carboxylic acid, NO and NO₂ concentrations were recorded above the acidic samples, while the methanol, ethanol, aceton and DMS concentrations are the means of the values obtained above the acidic, normal pH and alkaline samples.

The variation of the ammonia levels above the urine samples was not so marked as it was above the faecal samples. The kidneys naturally excrete ammonia into urine (with urea and creatine) (Davies et al., 1997) and so the presence of ammonia in the headspace was expected. If there is faecal contamination of the urine samples, the urea present in the urine is hydrolysed by the enzyme urease that is present in the faeces, producing ammonia and carbon dioxide (Robertson, 1994).

The relative concentration of the organic compounds was much lower above the urine samples than above the corresponding faecal samples. The concentrations of acetone and ethanol above the urine were very comparable to the concentrations of these compounds above human urine analysed by the same procedure (sample size and treatment) (Smith et al., 1999a).

Urine is sterile unless infected by disease and so we would not expect to see large concentrations of those species that are formed by bacterial action in the gut (notably the fatty acids). The concentrations of the fatty acids were indeed relatively low (compare Tables 1 and 2). The levels of pentanoic and hexanoic acids in the urine headspace were too low to be recorded in these measurements. The significant concentration of NO and NO₂ may be indicative of urinary infection, but this would need to be checked by culture tests.

That the DMS levels above these urine samples were much smaller than those above the faecal samples, suggests that the blood levels of DMS are small relative to the gut levels where the DMS is generated by the action of bacteria on sulphur-bearing proteins in the food (Prins, 1977). It is also worth to note that a molecule we tentatively identify as methanthiol, CH₃SH, appears in the headspace of the acidified urine at a low level of typically 100 ppb. This species is also known to be a product of protein metabolism by bacteria (Prins, 1977).

### 4. Conclusions

These initial experiments on the emission of volatile compounds from porcine faeces and urine demonstrate the value of SIFT/MS in this important area of animal science. In these experiments, the detection mass spectrometer of the SIFT/MS was scanned in the relatively low mass regime, the trace compound hexanoic acid being the largest molecule detected (molecular weight 116 Daltons). The vapour pressures of these organic molecules generally reduce with increasing molecular weight and so the reducing concentrations of the heavier carboxylic acids observed above the faecal samples (see Table 1) must, in part, be due to this effect. Larger faecal and urine samples and longer mass spectral acquisition times would surely reveal the presence of polar compounds with larger molecular weights, and different isomeric forms of some of the higher-order carboxylic acids that are known to be released from pig slurry (e.g., see O’Neill and Phillips, 1992). It would be very instructive to exploit the real-time feature of SIFT/MS by sampling the volatile emissions from faeces and urine immediately after excretion. In this way, the emission rates of the volatile compounds from excreta and the generation of organic compounds in slurry beds could best be determined. Thus, the pollution rate of the environment can better be assessed and ultimately controlled.

### Acknowledgements

The authors would like to thank Professor C.M. Wathes and Dr. T.M. Mottram (Silsoe Research Insti-
tute, Bedford) and Dr. R.B. Jones (Roslin Institute, Edinburgh) for their encouragement and support in all aspects of this research work and Dr. R.F. Parrott (Babraham Institute, Cambridge) for lending us the crates used to collect the urine and faecal samples. The authors would like to acknowledge partial financial support for this work provided by the North Staffordshire Medical Institute, the Biotechnology & Biological Sciences Research Council and the Grant Agency of the Czech Republic (203/97/P130). Two authors, DS and PS, would also like to thank The Royal Society of London for supporting their research collaboration with a Joint Project Grant.

References


