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Atmospheric pressure chemical ionisation mass spectrometry for in vivo analysis of volatile flavour release

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Abstract

To follow volatile flavour release in the expired air of people during eating, several physiological and analytical constraints must be observed to obtain good quality data. An interface has been developed to sample air from the nose and ionise the volatile compounds contained therein by atmospheric pressure chemical ionisation. The ions formed are detected in a quadrupole mass spectrometer. The interface design overcomes many of the constraints and allows volatile detection in the 100–10 ppbv range, depending on the nature of the volatile compound to be analysed. The principles and operating parameters of the interface are described. Control of the ionisation process is effected through the operating conditions within the interface. Since many compounds are introduced simultaneously into the interface, it is preferable to minimise fragmentation otherwise the spectra are extremely complex and it is difficult to assign ions to compounds unequivocally, with the result that quantification is imprecise. The ionisation parameters are set to favour formation of the protonated molecular (MH^+) ion from the compounds and minimise formation of cluster ions, which can also confuse the spectra. The sensitivity and linearity of the technique is demonstrated for a range of flavour volatiles with a dynamic range of three orders of magnitude. The lower limit of sensitivity is determined by the signal to noise ratio whereas the upper limit occurs when all the available charge is exhausted. The technique was designed primarily to monitor real-time changes in the concentration of known volatiles during eating and has limited identification power. However, for reliable quantification, it is necessary to assign the ions monitored in-nose with the volatile compounds present in the food. Besides assignments made on nominal mass, isotopic ratio analysis and accurate mass measurements have also been used to determine the contribution of certain compounds to a particular ion intensity.

Keywords: APCI

1. Introduction

1.1. Temporal aspects of flavour perception

Flavour perception is known to change during the time food is consumed. Most people can identify various temporal attributes of flavours, particularly aftertastes. The phenomenon is especially obvious in wine appreciation where a sequence of events contributes to the overall flavour perception. The initial sniff from the glass (the "nose" of a wine), the initial flavour as wine is placed in the mouth, and the subtle flavour changes after swallowing can be recognised by most people and described in great detail by trained wine tasters. The sensory study of the temporal aspects of flavour is embodied in the Time Intensity technique in which people record the intensity of a flavour with time (see Cliff & Heymann, 1993). Scientists have hypothesised whether the temporal dimension of flavour is due to differential rates of flavour release and transport of flavours from the food to the flavour receptors or whether it is a cognitive process. To try and answer these questions, various methods for measuring the release of flavours in vivo have been developed. Comparison of flavour release and Time Intensity sensory data may then determine which of the above hypotheses is true. Most methods are designed to follow the volatile concentration in-nose, as it is easier to collect and analyse volatile flavours compared to non-volatile flavours. Reviews of methods for monitoring volatile compounds

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in-nose have been published previously (Taylor, 1996; Taylor & Linforth, 1996, 2000), while methods for measuring non-volatile release have also been described (Davidson, Hollowood, Linforth & Taylor, 1999; Davidson, Linforth, Hollowood & Taylor, 2000; Davidson, Linforth & Taylor, 1998; Jack & Gibbon, 1995; Jack, Piggott & Paterson, 1995). The requirements of techniques for measuring flavour release in vivo are summarised in Table 1.

Conventional techniques for the analysis of flavour volatiles, like gas chromatography–electron impact mass spectrometry (GC–EIMS), separate the molecules first (GC) and then ionise the individual molecules so that they partially fragment and produce characteristic spectra from which the identities of the molecules can be deduced. While this gives detailed structural information, it is too slow for in vivo analyses (see Table 1) and not suitable for following release in real time. An alternative is to introduce the whole mixture of volatile compounds directly into the MS and resolve them entirely by mass. This gives real time analysis but at the expense of structural information and identification. It also places new demands on ionisation sources, many of which were designed to ionise single compounds, not a complex mixture of compounds at any one time. If mixtures of volatiles from foods are directly introduced into mass spectrometers, the ionisation sources also have to deal with the presence of air and water and some sources (notably Electron Impact) perform well below optimum under these conditions (Linforth & Taylor, 1993). For analysis of mixtures by MS, fragmentation of the compounds is actually a hindrance as the many fragment ions make interpretation of the data very difficult. Nonetheless, Hewlett Packard have introduced an indirect sampling EI–MS for volatile mixtures where the complex mass spectral patterns are analysed by software so that the patterns can be related to individual samples. The technique seems to be more qualitative than quantitative. For quantitative analysis, soft ionisation techniques, like chemical ionisation, are preferred. In our opinion, techniques involving ionisation based on proton transfer [either atmospheric pressure chemical ionisation (APCI) or proton transfer reaction (PTR)], followed by mass spectrometry, are the best current options for volatile flavour analysis. Both techniques can cope with water and air, produce single ions for most compounds and operate at pressures, which allow easy and safe sampling of air from people to the source. In this paper we describe in detail the interface developed in our laboratory for APCI–MS analysis of flavour volatiles and the way in which it is operated and calibrated.

1.2. Fundamental principles of atmospheric pressure chemical ionisation

APCI sources are simple in terms of construction, consisting of just an inlet and an ionising source, which can be a radioactive isotope or a corona discharge. The latter are favoured in typical laboratory scale instruments due to the greater dynamic range. Ions formed are sampled into a standard quadrupole MS maintained under vacuum. The APCI process involves the formation of an initial reactant ion, as the reactant molecules travel through a point-to-plane corona discharge at atmospheric pressure (Raffaelli, 1997). The reactant ion can then transfer its charge to any molecule [R; Eq. (1)] with a higher proton affinity. Water is an excellent choice for the reagent molecule (see Table 2) as its proton affinity lies between those of the main components of air (nitrogen, oxygen and carbon dioxide), but below that of most volatile organic compounds. This has

Table 1
Requirements of techniques for successful measurement of flavour release in vivo

Factor	Physiology/Flavour considerations	Analytical limit
Speed	Breathing cycle Once every 5 s	Need 50–500 data points to see detail Sampling every 0.1–0.01 s
Sensitivity	Odor threshold Depends on individual compounds	ppmv to pptv (ul/l–pl/l) Linear response Dynamic range of several orders of magnitude
Response	2600 Volatile compounds in foods	Universal detection Capable of simultaneous detection
Air	Volatiles present in expired air	Not affected by common constituents of air
Water	Humid air in nose and mouth Headspace above foods also contains water vapour	Up to 100% RH
Human interface	Must not interfere with normal eating and breathing patterns	Ethical considerations Interfacing people to high vacuum of MS

Table 2
Proton affinities of some components of air and common volatile flavour compounds (values taken from Hunter & Lias, 1998)

Compound	Proton affinity (kJ mol^{-1})
Air components	
Nitrogen	493.8
Oxygen	421
Carbon dioxide	540.5
Argon	369.2
Methane	543.5
Water	**691**
Acids	
Formic acid	742
Acetic acid	783.7
Propionic acid	797.2
Butyric acid	804.9
Pentanoic acid	806
Alcohols	
Methanol	754.3
Ethanol	776.4
2-Propanol	793.0
Propanol	786.5
Butanol	789.2
2-butanol	793.7
Tert-butanol	802.6
Pentanol	806
Aldehydes	
Acetaldehyde	768.5
Propionaldehyde	786.0
Ketones	
Acetone	812
Diacetyl	801.9
3-Hexanone	843
Esters	
Ethyl formate	799.4
Methyl acetate	821.6
Methyl butanoate	845
Sulfides	
Methyl sulfide	773.4
Dimethyl sulfide	830.9
Ethyl sulfide	789.6
Dimethyl disulfide	815.3
Propylthiol	794.9
Amines/amides	
Acetamide	863.6
Methylamine	899.0
Dimethylamine	929.5
Trimethylamine	948.9
Heterocycles	
Thiazole	904
Furan	803.4
Thiophene	815.0
Pyrazine	877.1
Pyrrole	875.4
2-Methylthiazole	930.6
γ-butyrolactone	840.0

several advantages. First, water is a requirement for the analysis and not an obstacle. Secondly, none of the air components are ionised but the wide range of organic compounds, which possess odour, are susceptible to ionisation under suitable conditions. There are detailed

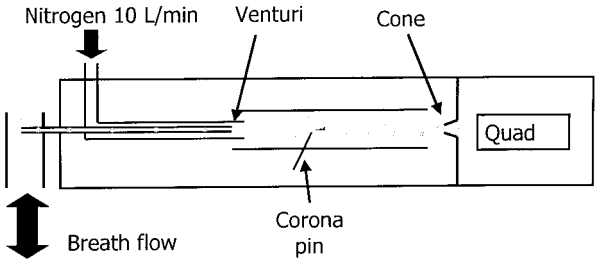

Fig. 1. Schematic diagram of the API source.

descriptions of the proton transfer process in the literature (Eiceman & Karpas, 1994; Hansel, Jordan, Holzinger, Prazellar, Vogel & Lindinger, 1995; Howe, Williams & Bowen, 1981).

$$H_3O^+ + R \rightarrow RH^+ + H_2O \quad (1)$$

APCI ionisation is "soft" meaning that the reagent ions have sufficient energy to ionise the molecule but not to fragment it to any great degree; thus most compounds produce RH^+ ions. Alcohols dehydrate to give $[M - H_2O + H]^+$ as do aldehydes, although not to the same extent, and the degree of fragmentation can be controlled somewhat by altering the cone voltage (see Figs. 1 and 3 and discussion below on the interface). The spectra from APCI show few fragment ions and most ions can be associated with a single compound although, when isobaric compounds are present, separate identification by GC–MS, or some other means, is required.

However, APCI is a difficult process to control and some literature reports describe non-quantitative results and selectivity in the ionisation of particular compounds. Eiceman and Karpas (1994) reviewed some of the problems with APCI in Chapter 2 of their book and cite the work of Sunner (Sunner, Ikonomou & Kebarle, 1988a; Sunner, Nicol & Kebarle, 1988) who suggested that the ionisation of compounds depended on their gas phase basicity. This is a measure of the ability of a compound to accept a proton and values for some 1700 compounds can be found in Hunter and Lias (1998). Sunner, Nicol et al. (1988) determined the relative sensitivity for a wide range of molecules and found that, in their APCI system, compounds with a gas phase basicity above 200 kcalmol^{-1} gave similar responses while below 200 kcalmol^{-1}, there was a variable, but roughly linear response. Some compounds (dimethylsulfide, thiophene and furan) gave no signal at all. Sunner, Nicol et al. 1988 used relatively high water concentrations (relative humidity in source 21%) and thus formed water cluster ions of the general form $H_3O^+.(H_2O)_n$ where n could be 4–7. These ions can also transfer charge to analyte molecules making the overall ionisation complex and non-quantitative for some molecules. Inherent in this situation is the idea that one molecule

with a strong affinity for protons can become selectively ionised and "steal" the charge from other molecules with a lower affinity for protons. The result is that the extent of ionisation depends on the other molecules present in a mixture making the process variable and incapable of quantification. This is obviously of no use for an analytical technique.

The Sunner papers are widely quoted but there are others that show excellent quantification for mixtures of volatiles, for instance the work of Ketkar, Penn and Fite (1991) who monitored selected volatiles in the chimney of an incinerator down to a few ppt. The reasons for the differences lie in the way that the APCI source is operated and Eiceman and Karpas (1994) summarised these as:

(a) control of the amount of water entering the source and (b) the temperature at which the source is operated.

A third important factor, which is not so well-documented, is the process of declustering. In many APCI sources, a stream of dry nitrogen (often referred to as the curtain gas) is positioned between the source and the quadrupole entrance. Sunner, Nicol et al. (1988) concluded that declustering occurred due to the low water content of the curtain gas as well as collision-induced declustering as the clusters interacted with the fast flow of gas and were accelerated through the sampling orifice into the high vacuum region of the mass spectrometer.

Our approach has been to control conditions within the APCI source by regulating the water concentration in the source, maintaining a suitable and constant source temperature and limiting cluster ion formation so that the APCI source can be operated under quantitative conditions. At this point it is appropriate to mention the alternative approach developed by Lindinger and coworkers (see for example Lindinger, Hansel & Jordan, 1998a,b) which they have named proton transfer reaction (PTR)–MS. To overcome the problems mentioned above, they have generated the reagent ion (H_3O^+) separately and then controlled the reaction between reagent ions and volatile organic compounds in a specially developed drift tube operated at precise temperature and pressure. The result is that the rate constants for different volatiles in the proton transfer process are all very similar (1.3 to 4.7×10^{-9} $cm^3.\ s^{-1}$ Lindinger et al., 1998a) and, therefore, all components in a mixture will be ionised to the same extent (Lindinger, personal communication). While PTR–MS has the advantage of precise control over ionisation, the sensitivities reported for flavour volatiles are similar to those obtained with APCI. The simplicity of the APCI process is also lost. PTR–MS is not yet widely available. Spanel and Smith (1999) have also developed a similar PTR–MS technique but using a selected ion flow tube. Their spectra are characterised by water and analyte cluster ions (Spanel & Smith, 1999) which makes interpretation difficult (Taylor & Linforth, 2000) and sensitivity seems to be lower than with the Lindinger technique.

1.3. APCI as a gas phase analyser

APCI was originally developed as an analytical technique for analysis of trace components in the gas phase. Early attempts at a breath interface (Benoit, Davidson, Lovett, Nacson & Ngo, 1983) were not entirely successful although the TAGA (trace atmospheric gas analyser) based on APCI with a triple quadrupole mass spectrometer (built by Sciex and now part of Perkin Elmer) was applied to many other trace gas analyses with great success. We were attracted to APCI due to its simplicity, its sensitivity (ppt levels; Benoit et al.), its ability to work under difficult conditions and the robust nature of the apparatus. Our overall aim was to build an interface that would allow breath-by-breath release of flavour volatiles to be monitored in-nose so that real-time flavour release from foods could be monitored. Taking into account the factors listed in Table 1, the key requirements were that:

- the interface should allow collection of expired air from people during eating without interfering with their normal eating or breathing patterns;
- ionisation should be controlled to produce "clean" spectra with little fragmentation to aid interpretation of the data;
- the speed of data collection should be sufficient to see detail of volatile release on a breath by breath basis;
- response should be linear with a lower limit in the ppb range;
- interactions between analytes should be minimised to allow quantitative analysis of a component in a mixture;
- a calibration procedure should be developed to express volatile concentrations on a $mg.m^{-3}$ or ppbv ($nl.l^{-1}$) basis.

2. Material and methods

The interface design has been published in a European Patent application (Linforth & Taylor, 1998) and in a US patent (Linforth & Taylor, 1999). Details of construction and operating parameters are given there. Here some of the details of the device are given and a schematic view is shown in Fig. 1.

3. Results and discussion

3.1. Air sampling

The sampling of air from the nose of people needs to meet ethical standards for human experimentation and

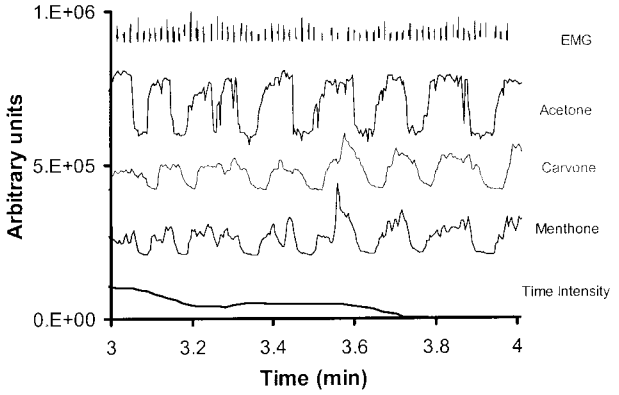

Fig. 2. Breath by breath concentrations of volatiles in nose during the eating of chewing gum. The EMG (electromyography) trace shows mouth movements, acetone is a marker for exhalation, carvone and menthone are markers for the release of mint aroma and the time intensity trace was obtained from a trained panellist who monitored the intensity of minty flavour with time. The signals were aligned using reference marks.

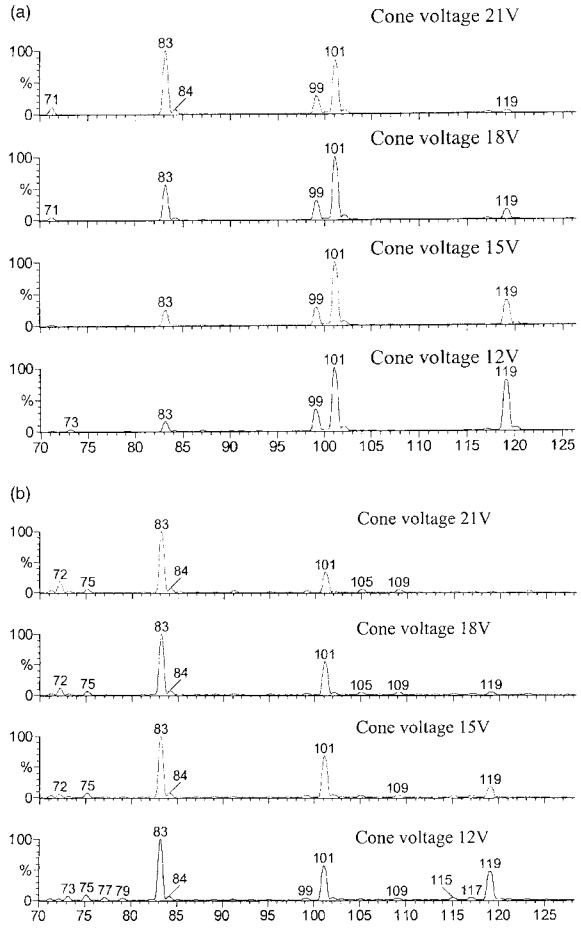

Fig. 3. (a). Effect of cone voltage on fragmentation and ion intensity of hexanal (MW 100). Ion 101 is the protonated molecular ion [MH^+]. Ion 83 is the protonated, dehydrated molecular ion [$M\text{-}H_2O+H^+$]. Ion 119 is a cluster ion [$M+H_3O^+$]; (b). Effect of cone voltage on fragmentation and ion intensity of hex-3-enol (MW 100). Ion 101 is the protonated molecular ion [MH^+]. Ion 83 is the protonated, dehydrated molecular ion [$M\text{-}H_2O+H^+$].

this involves both hygiene and minimum invasion. To this end, small disposable plastic tubes (made by cutting the tip from plastic pipette tips of approximately 10 mm diameter and 40–50 mm length) were inserted in one nostril so that assessors could breath, drink and eat normally. Air was sampled orthogonally through a 0.53 mm diameter deactivated fused silica capillary tube that was pushed through a hole made in the middle of the pipette tip. The flow rate in the capillary was between 10 and 50 ml min^{-1} depending on the amount of compound in the exhaled air. On exhalation, the small plastic tube extension to the nostril was filled with expired air and the fused silica capillary sampled only exhaled air. On inhalation, the tube was filled with laboratory air, which was then sampled into the fused silica capillary. Thus the tidal flow of inhalation and exhalation was monitored and the APCI-MS traces showed breath by breath volatile concentrations.

Flow was achieved through a venturi made by sheathing the deactivated capillary in another tube carrying a flow of nitrogen at 10 l min^{-1} (see Fig. 1). At trace levels, a significant proportion of the volatile sample can be lost on active surfaces like pump seals or on ferrules and by using a single piece of deactivated fused silica tubing, losses were minimised. The silica tubing was heated (100°C) to prevent water or volatile condensation. The dead volume of the tubing was low (about 75 mm^3) and the relatively high flow rate meant there was an insignificant delay in response time when a volatile was introduced at one end of the sampling system. For headspace analyses, the fused silica was placed directly into the headspace. The thermal mass of the transfer line and the end fittings maintained the tip of the capillary at elevated temperature to avoid any condensation of volatile at this point.

To ensure that assessors were indeed breathing through their nose during eating, and to check the regularity of their breathing, the concentration of acetone was routinely monitored. Acetone is a metabolic product from the liver, is transported in blood and crosses the blood–air interface in the lungs. It is, therefore, a marker for exhalation and can indicate whether volatiles are entering through the ortho- or retro-nasal routes during consumption of food. With beverages, for instance, volatile might enter the nose orthonasally from the headspace above the beverage, as well as from the beverage in mouth via the retronasal route. Close examination of breath-by-breath traces, using acetone as the exhalation (or retronasal route) marker, can give some estimate of the relative importance of the retro and ortho-nasal routes.

Although the breathing cycle takes around 5 s, chewing movements actually pump a small amount of headspace from the mouth into the throat and, to see these details the concentration in-nose needs to be monitored rapidly. Since the dwell time (the time spent by the mass

spectrometer in monitoring any one ion) has little effect on APCI sensitivity, each ion is monitored for 0.01–0.05 s. If five ions are monitored simultaneously, then each ion will be sampled every 0.05 or every 0.25 s respectively. An example of the detail that can be seen with this speed of sampling is shown in Fig. 2, where volatiles, sensory time intensity and chewing frequency have been collected simultaneously from a subject chewing gum. The pumping action of the mouth can be seen in the small jagged peaks on each breath in the carvone and menthone traces. Closer examination shows that many of the chews (shown on the EMG trace) are aligned with transient decreases in acetone concentration (a small negative peak) and an increase in the carvone and menthone concentrations (small positive peaks).

3.2. Control of ionisation

From the discussion above, it is clear that the water content in the APCI source needs to be regulated so that the ionisation process is constant. If exhaled and inhaled breath were the only source of water in the APCI source, the water content would change from around 100% relative humidity (RH) on exhalation to between 30 and 70% (typical ambient air RH in temperate climates) on inhalation. Similarly if hot beverages are sampled then the headspace above the beverage will be saturated with water and the situation will be even more challenging. To overcome these fluctuations in water content, we used the venturi nitrogen flow of 10 $l.min^{-1}$ as a "buffer". Sample flow is between 5 and 50 $ml.min^{-1}$ and is insignificant compared to the venturi flow with the result that the water content of the nitrogen gas used to drive the venturi, determined water content in the source. High purity cylinder nitrogen was used initially which has a quoted water content of less than 10 ppm. Occasionally, we received cylinders with high water contents, such that the ionisation spectra contained many water ion clusters. Nitrogen is now generated from a compressed air source and any hydrocarbons are removed through a zero air generator. The system produces sufficient nitrogen to run two APCI-MS systems with a water content in the low ppm range. Although there may be minor fluctuations from day to day, the system is calibrated with authentic compounds for each run and any variation in ionisation will be accounted for when the ion intensities are converted into concentrations of volatiles. In practice, a spectral scan shows the presence of H_3O^+ at m/z 19 with another peak at m/z 37 due to $H_3O^+H_2O$.

Temperature within the source is controlled by heating the source block and by heating the incoming nitrogen venturi gas. Without pre-heating, the flow of nitrogen cools the source quite effectively. The aim is to maintain the source temperature at 50°C.

The high flow of nitrogen creates an extremely turbulent flow within the source, which has a volume of around 30–50 ml. This flow has a major effect on sensitivity and we have shown (unpublished data) that reducing the nitrogen flow actually decreases the sensitivity of the APCI source. The reason for this is not clear but

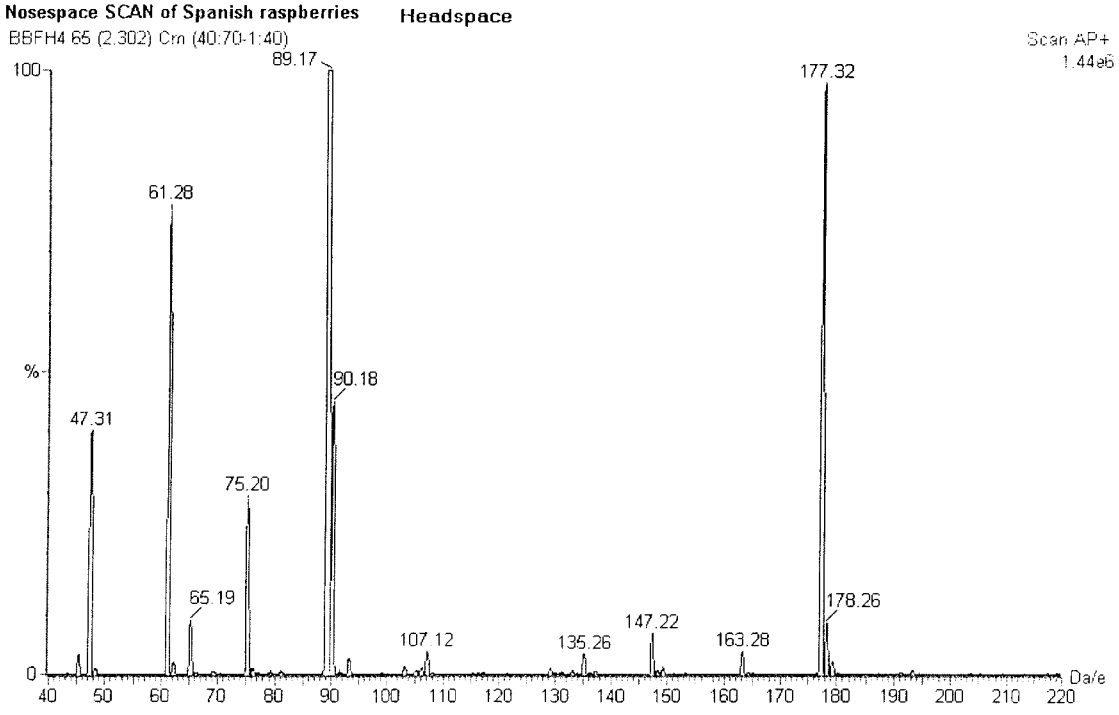

Fig. 4. Headspace from fresh raspberries showing the "clean" spectra obtained using APCI-MS.

could be due to a transport effect (ions are physically transported to the sampling orifice of the MS) or due to effective declustering of ions so that the signal is focused into the ion being monitored (the MH^+ ion).

As noted previously, APCI causes little fragmentation but, to optimise sensitivity, it is preferable to form one ion rather than two as this increases the signal to noise ratio. Fragmentation can be controlled to some extent by altering the voltage on the sampling cone (the cone voltage). In contrast, the corona pin voltage has no significant effect on fragmentation and is maintained at 4 kV for all compounds. With the Micromass Platform and Platform LCZ machines (Altrincham, UK), it is possible to set up ionisation parameters for each compound (in selected ion mode) and a library of parameters for common volatile compounds has been assembled in our laboratory. Headspace containing authentic compounds is introduced into the APCI source while the cone voltage is changed and the ions formed are monitored. For most compounds, the voltage that gives the greatest ion intensity for the MH^+ ion is used. For compounds that fragment, or where there are compounds with identical mass, it is worth sacrificing some sensitivity to be able to discriminate the compounds on the basis of their different ion patterns. An example is given in Fig. 3. for hexanal and hexenol both of which have MW of 100. Hexanal ionises to give a strong 101 ion at low cone voltages, whereas hexenol can be dehydrated to the $(M - H_2O + H)^+$ ion at m/z 83. Table 3 contains some typical examples of cone voltages and fragment ions for some volatile flavour compounds. The result of this optimisation is a "clean" spectrum where each compound produces one major ion. Fig. 4 shows headspace analysis of fresh raspberries with the MS set in scan mode and the major volatile components can be clearly seen.

3.3. Mode of MS operation

Since the purpose of in-nose APCI monitoring is to follow the sensory signals received at the olfactory receptors, it is logical to monitor those compounds that have a major sensory impact. For any given food sample, character impact compounds may be known from the literature or from separate GC-Olfactometry experiments. Ideally, one would want to choose all the compounds that contribute to the sensory character and monitor them simultaneously in-nose. In practice, this ideal can rarely be met as some compounds have very low thresholds and lie outside the detection limits of APCI. One way around this problem is to choose a range of volatiles from the food which represent the physicochemical parameters, important in flavour release (e.g. volatility and hydrophobicity). If a suitable range does not exist naturally, a mixture of compounds can be added to some foods to act as markers for flavour release. By following the release of these compounds (which are present at sufficient concentrations for analysis by APCI) models for release can be constructed and the behaviour of compounds that cannot be analysed directly can be predicted. Linforth, Friel and Taylor (2000) built a model to describe the observed release behaviour of twenty five compounds from gelatin-sucrose confectionery gels. From the model, the concentration of any compound in-nose could be predicted from its structure and concentration within the gel, with logP and volatility being two of the key parameters. The predictive power was adequate to forecast the behaviour of a second test set of compounds. Although this method was developed to overcome the sensitivity limits of APCI, it is a convenient way of predicting release behaviour and is being applied to other flavour release situations.

Table 3
Optimum cone voltage and fragment ions for some volatile flavour compounds

Compound	Cone voltage (V)	Major ion (m/z)	Minor ions >5% m/z value (percent)
Dimethylsulfide	20	63	47 (8)
Amyl acetate	18	131	61 (23)
Diacetyl	20	87	None
2,5-Dimethylpyrazine	30	109	None
Furfural	20	97	None

3.4. Linearity of response

For quantification of compounds, the relationship between instrumental response and concentration needs to be determined. Fig. 5 shows typical calibration curves for some volatile compounds on APCI. The upper limit of sensitivity is set when all the available charge is

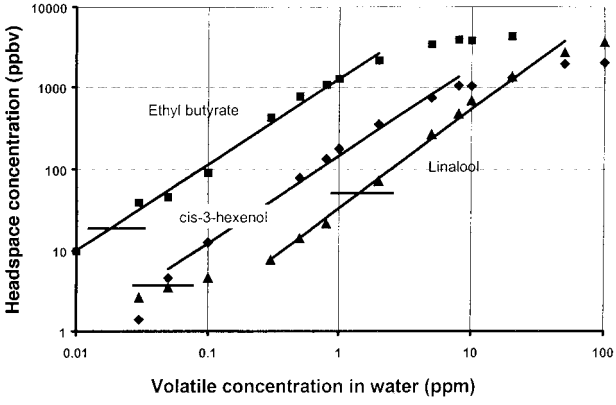

Fig. 5. Calibration curves for three flavour volatiles showing the linear response and the limits of detection at high concentrations (due to limiting reagent ion) and at low concentrations (due to low signal to noise ratios). The horizontal bars on each calibration show the published odour thresholds for the compounds.

utilised by the volatiles and there is no increase in signal with increasing volatile concentration. This can be confirmed by watching the intensity of the water ion at m/z 19 decrease to zero under these conditions. The lower limit is set when the signal to noise ratio decreases below a ratio of 3:1. In between, the response is linear over about three orders of magnitude on the Platform II machine fitted with the prototype interface used for these experiments. The newer Micromass Platform LCZ equipped with the production version of the interface gives some improvement in the dynamic range. Although some compounds have very low odour thresholds, the current sensitivity of the APCI technique can detect many compounds at, or near their thresholds and the horizontal bars in Fig. 5 indicate the corresponding odour threshold values for the three compounds.

The noise in APCI is a feature of the technique and sensitivity improvements are made both by enhancing the signal (using the cone voltage technique described above) and by reducing the background. Using high purity nitrogen in the venturi assists in background reduction and we have found the use of a large air scrubber (filled with activated charcoal and permanganate) beneficial in reducing the level of background volatiles in the laboratory air. These volatiles tend to come from plastic fixtures, paint and from plastic flooring materials.

3.5. Suppression of signal

In the Introduction, the concerns over selective ionisation of some compounds were discussed. There are some foods where one particular volatile component may be present at much higher levels than the other volatiles and, potentially, the major component might suppress ionisation of the minor components. Alcoholic beverages are good examples with beer containing around 4–5% ethanol and wine containing around 12% ethanol. Beer has been successfully analysed by headspace and by in-nose measurements in our laboratory (unpublished data). Data analysis in these situations needs to take into account the ions formed from ethanol since they make a significant contribution to the spectrum when ethanol is present at percent levels. A solution of ethanol was prepared at the same concentration as found in the beer and the spectrum obtained from this solution was subtracted from the beer spectrum so the minor components could be more clearly seen. Fig. 6 shows the headspace profile above a commercial beer after subtraction of the ethanol signal. The ester series can be clearly seen along with benzaldehyde. Using this method, it was possible to quantify the minor volatile components in beer. Rigorous experiments with wine have not yet been undertaken although preliminary headspace measurements of wine show the expected volatiles as well as ethanol.

Sunner, Nicol et al. (1988) suggested that ionisation in mixtures might be affected if one component had a greater affinity for protons than another (selective suppression). It is relatively simple to test for suppression by analysing mixtures of volatile compounds. To test our APCI interface, a mixture of seven compounds was prepared which exhibited a range of proton affinities. Although precise values were not available for 2,5-dimethylpyrazine nor for acetylthiophene, the values for the base compounds (pyrazine and thiophene) are known and these two compounds possess proton affinity values near the top end of the range (>900 kJ mol^{-1}). If selective suppression was occurring, one might expect that the ion intensities for compounds with low proton affinities would be decreased, either partially or completely and charge would be preferentially taken by the pyrazine and thiophene derivatives. To investigate this possibility, an aqueous solution of the seven compounds was prepared in a sealed bottle (at concentrations typically found in food; Table 4) and allowed to equilibrate with the headspace. A sample of headspace was analysed by APCI and concentrations in the gas phase calculated after calibration with authentic standards. The air-water partition coefficients for each compound were then determined and compared with values obtained by calculation (Marin, Baek & Taylor, 2000). If selective suppression was occurring, ionisation of compounds with proton affinities close to that of water (691 $kJmol^{-1}$) should be suppressed and the headspace concentration would be underestimated leading to a smaller K_{aw} value. However, the experi-

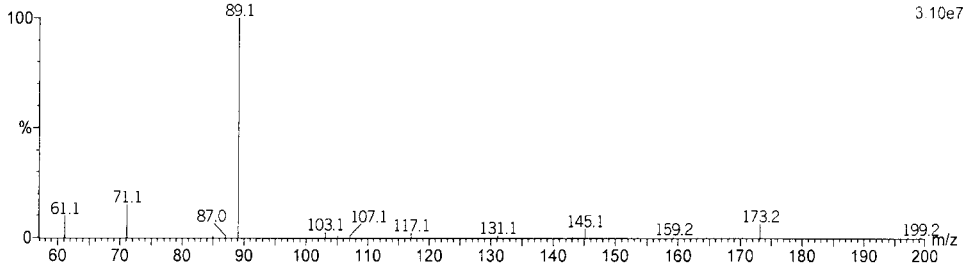

Fig. 6. Headspace spectrum of a commercial beer after subtraction of the ethanol signal. Tentative identities; 61 acetic acid; 89 ethyl acetate; 105 benzaldehyde; 117 Ethyl butyrate; 131 isoamylacetate; 145 ethyl hexanoate; 173 ethyl octanoate.

Table 4
Properties of seven volatiles in aqueous solution and their air water partition coefficients determined after experimental measurement of the equilibrium headspace concentration and by calculation. The close agreement between the experimental and calculated K_{aw} values indicates that, despite a range of proton affinity values, all compounds are adequately ionised in this mixture and quantitative analysis is achieved. Proton affinity of water 691 kJmol^{-1}

Compound	Concentration in water mg kg^{-1}	Concentration in headspace μgl^{-1}[a]	Proton affinity KJ mol^{-1}[b]	K_{aw} at 25°C	
				Calculated $\times 10^3$[c]	Experimental $\times 10^3$
Acetaldehyde	1.1	3.2	768.5	2.9	2.7
Dimethylsulfide	0.846	68	830.9	81	25
Diacetyl	0.961	0.55	801.9	0.57	0.39
(E)-2-hexenal	0.11	0.19	No data	1.7	2.6
2,5-Dimethylpyrazine	8.37	0.53	877 (Pyrazine)	0.063	0.057
2-Acetylthiophene	2.04	0.17	815 (Thiophene)	[d]	0.082
Menthone	0.0893	0.62	No data	6.9	7.1

[a] Concentration in water multiplied by calculated K_{aw} (except for acetylthiophene where experimental value used.
[b] Hunter and Lias (1998).
[c] $P^0_{water}(25°C) = 3123$ Pa.
[d] Activity coefficient for 2-acetylthiophene was not available.

mental K_{aw} value for acetaldehyde (the component with a proton affinity closest to that of water) agrees well with the value calculated from thermodynamic data in the literature. Even the value for dimethylsulfide (a compound not ionised in the Sunner APCI source) is within the range reported in the literature (See for example the K_{aw} values for dimethylsulfide Van Boekel & Lindsay, 1992). Overall, the two sets of partition coefficients agree quite closely. The conclusion is that, under the operating conditions of our APCI interface and using these concentrations of volatiles, selective suppression does not occur so that volatiles can be quantified with confidence. However, given the potential for suppression, we recommend carrying out tests to ensure the validity of data obtained under specific operating conditions.

3.6. Calibration

To convert measured ion intensities into concentrations, the interface is fitted with a calibration port so that authentic compounds can be introduced into the gas stream. Preparing dilutions of volatiles in the gas phase is extremely difficult and our approach has been to use solutions of volatiles in hexane or cyclohexane. These can be prepared with considerable accuracy and then introduced via a microsyringe on a syringe pump into the heated flow of venturi nitrogen, which is running at 10 l min^{-1}. The volumes of solution introduced per unit time are very small (μl of solution) and the fast flow of gas, plus the elevated temperature, volatilise the compounds instantly. By using dilutions of a stock solution, calibration curves like those in Fig. 5 can be produced and the concentration of volatiles in the sample stream calculated since the flow rate of the sample can be accurately measured with a flow meter. Although cyclohexane has a proton affinity lower than that of water (Hunter & Lias, 1998) some low intensity ions are still observed in APCI-MS that suggest ionisation of this compound is occurring. The proton affinity value for hexane is not listed in Hunter and Lias but low intensity ions are also seen for this compound.

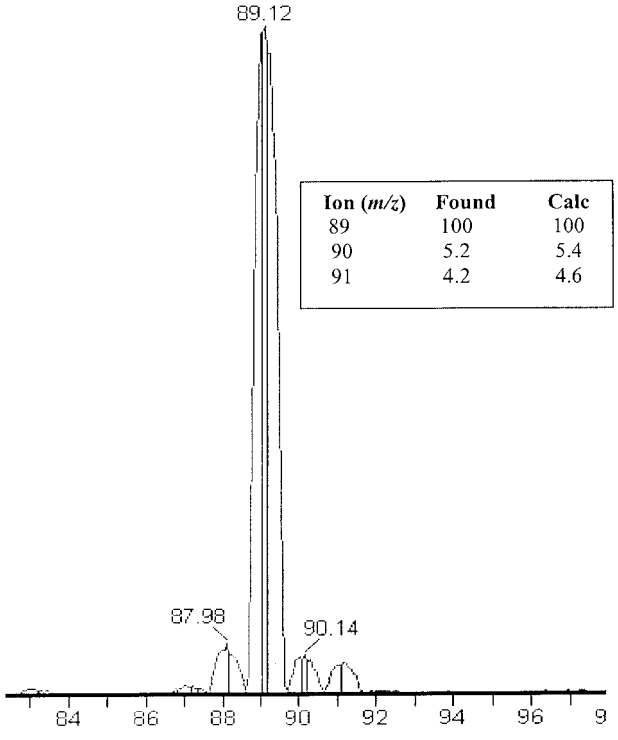

Fig. 7. Identification of allyl methyl sulfide by isotopic ratio analysis. The ions at 90 and 91 are due to the natural isotopes of sulfur.

3.7. Assigning ions to compounds

As explained in the preceding sections, ionisation of volatile mixtures by APCI-MS is performed under controlled conditions so that each component in the volatile mixture, ideally produces one characteristic ion from which it can be quantified. However, the minor fragment ions from some compounds (Table 3) may occur at the same m/z value as another compound and thus the contribution of each compound to the ion intensity needs to be determined. When compounds with the same mass are analysed, it is not possible to differentiate them and thus positional isomers like 2- and 3-methylbutanal can only be measured as "methylbutanals". A similar situation exists for the terpene families where there are many positional- and stereo-isomers with the same molecular formula which can only be described by APCI-MS as monoterpenes, sesquiterpenes etc. In these cases, GC–MS analysis of the volatile compounds can give the composition of the terpene fractions. If one assumes that stereo and positional isomers are released in the same way because their physicochemical properties are similar, one can estimate the composition of isomers within a particular group of compounds. This is not entirely satisfactory and a direct measurement of each compound would be preferable. For our academic research on flavour release, we tend to choose volatile molecules that avoid these problems. For analysis of real flavours however, some more sophisticated solutions are needed.

Cone voltage can be used to differentiate some compounds with the same molecular mass, but with different chemical properties, and the example of hexanal and hexenol was shown in Fig 3. For some compounds, isotopic ratios can be used to confirm whether the intensity of a particular ion contains contributions from some other compound. In the analysis of volatiles from garlic (Taucher, Hansel, Jordan & Lindinger, 1996), isotopic ratios were used to confirm the identity of allyl methyl sulfide in PTR-MS. The same technique can be

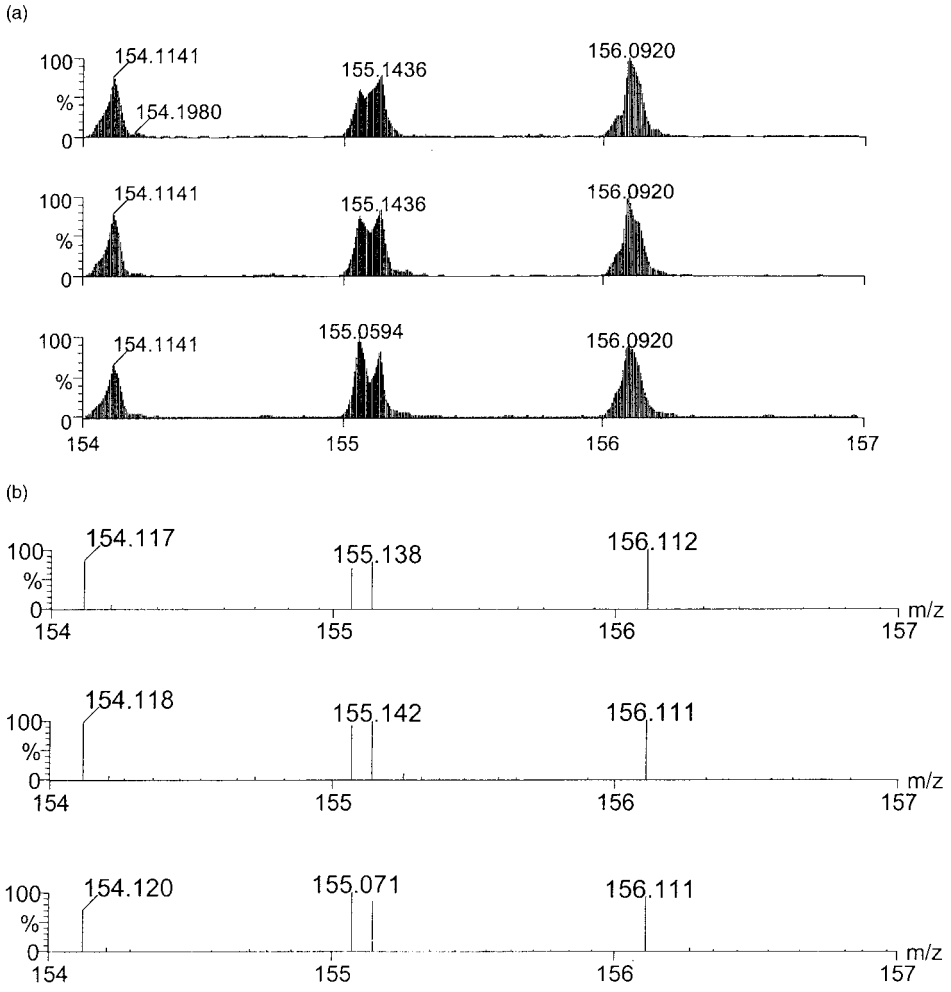

Fig. 8. (a) Time of flight mass spectrometry of coffee headspace. Each of the three traces shows the spectrum at a different cone voltage, demonstrating that there are two compounds around m/z 155; (b) time of flight mass spectrometry of coffee headspace. Using p-cymene as lock ion, accurate mass data for the two components around m/z 155 can be obtained. Each trace was obtained at a different cone voltage.

applied with APCI-MS and Fig. 7 shows the isotope distribution and ratios for allyl methyl sulfide where the experimental and theoretical ratios are in close agreement indicating that this is the only compound contributing to the ion intensity at m/z 89, 90 and 91. This technique is, however, unreliable for mixtures of volatiles.

Besides isotopic ratios, accurate mass measurements can be used to differentiate compounds with the same nominal mass but with different elemental compositions. For instance the compounds $C_6H_{10}O$ and $C_5H_6O_2$ both have a nominal mass of 98 but their accurate masses are 98.0732 and 98.0368 respectively. To investigate the feasibility of this technique, the APCI interface was connected to a time of flight (TOF) MS and coffee headspace was introduced. Coffee contains several hundred volatile components and thus represents a serious challenge for this type of analysis. In the headspace spectrum a mass peak around m/z 155 was identified which appeared to contain two compounds. By changing the cone voltage, two peaks could be clearly discerned (Fig. 8a). Using para cymene as a reference on which to "lock" the mass, it was possible to determine accurate masses for the two components of the peak (Fig. 8b). With a mass difference of 25 mDa two compounds can be discerned; at 40 mDa, the masses can be resolved. APCI-TOF-MS offers the benefits of accurate mass analysis and improved sensitivity when more than 10 compounds are analysed simultaneously. Below 10 compounds, quadrupole detection is equally sensitive (unpublished data from our lab). However, because of the way that TOF operates, it is not possible to use a specific cone voltage for each compound and so the spectra are not as clean as those produced using APCI-quadrupole-MS.

4. Conclusion

APCI-MS provides a technique for following volatile release *in vivo* on a breath by breath basis. It has its strengths and weaknesses but has yielded much useful data about flavour release. It has provided information on the link between flavour release and sensory perception (Baek, Linforth, Blake & Taylor, 1999; Linforth, Baek & Taylor, 1999) which suggests that the rate of release is an important factor in our perception of flavour intensity. Comparison of flavour release from regular and reduced fat products has produced quantitative data to show the effects of changing fat levels (Brauss, Balders, Linforth, Avison & Taylor, 2000; Brauss, Linforth, Cayeux, Harvey & Taylor, 1999). In fruits which generate some of the flavour compounds only on maceration, APCI-MS is fast enough to follow the enzymatic process in real time, allowing analysis of individual fruits in around 3 min (Brauss, Linforth & Taylor, 1998). The monitoring of labile compounds that cannot normally be seen in conventional GC-MS analyses has also been reported. Propene sulfenic acid was monitored in the headspace of garlic (Dunphy, Boukobza, Chengappa, Lanot & Wilkins, 2000) and 2-acetyltetrahydropyridine was found in the headspace above hydrated crackers (Grab & Gfeller, 2000). The ability to measure rapid changes has opened new opportunities for following the changes that occur when food packages are opened and indicated some of the key physicochemical factors involved (Marin et al., 2000). It has also allowed new models for flavour release to be constructed (Linforth et al., 2000) and the validation of existing models. The availability of techniques to measure both volatile and non-volatile release is shedding light on the interactions of these classes of flavour compounds (Davidson, Linforth & Taylor, 1998; Davidson, Hollowood et al. 1999; Davidson, Linforth et al., 2000). With further advances in MS technology we can expect to see more powerful, smaller and, hopefully cheaper machines available in the future. These may be applied to on-line monitoring of food processes, in environmental monitoring or in medical diagnostics.

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