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Review article

Real time breath and headspace analysis of flavour volatiles

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Abstract

An overview is presented of the principle, scope and major applications to date of the use of atmospheric pressure chemical ionisation mass spectrometry (APCI-MS) for monitoring the kinetics of release of flavour volatiles in real time, principally from breath during eating. The technique is rapid, quantitative, sensitive to the ppb level and can be used to monitor the vast majority of flavour volatiles. Advances made during the last 5 years in our understanding of factors affecting flavour release, particularly when conducted simultaneously with sensory evaluation, are contributing increasingly to more efficient product development in the food and flavour industry and to the design of flavour systems with desired dynamic flavour characteristics. Real time APCI-MS headspace data may be used to validate mathematical modelling of flavour release. It is proposed that these advances may be applied with similar benefits in the pharmaceutical industry, particularly in the improvement of the flavour acceptability of orally administered drugs.

Keywords: Breath analysis; Flavour volatile; APCI-MS; Headspace; Mathematical modelling

1. Introduction

Research using a new instrumental technique in the food and flavours industry into factors affecting flavour release from foods and beverages is enabling scientists to contribute to the development of flavour systems with desirable dynamic release characteristics, and thereby to enhance consumer preference. The flavour impression of orally administered solid dosage forms (orodispersible and chewable tablets, flavour-coated tablets) is similarly a major factor in patient acceptance and compliance. The same is true of liquid dosage forms (syrup, sachet, hot drinks, effervescent tablets) where off-notes from undesirable chemicals may further reduce patient acceptance. Flavour impression is not only a key factor for product selection in the very competitive OTC market, but also in the prescription market where unpleasant taste (mostly bitterness) may be a major issue for children [1] and the elderly [2].

The technique described in this paper uses an advance in atmospheric pressure chemical ionisation mass spectrometry (APCI-MS) for the detection of gas phase volatiles. It is rapid, quantitative, sensitive to the ppb level and can be used to monitor the vast majority of flavour volatiles. The greatest advantage, however, is its capability to monitor flavour volatiles in real time. Although volatiles in any gas phase sample can in principle be monitored, a particularly valuable application is the determination of the dynamics of flavour release into the breath during eating or taking any orally administered drug. Release profiles of flavour volatiles into the breath typically differ greatly from those arising from the same source in any experimental arrangement in vitro owing to the complex dynamic processes occurring in the mouth. Thus an objective measure is readily achieved of absolute flavour volatile concentrations in the breath, and their variation with time, during ingestion of foods or orally administered formulations. Comparisons may be made, for example, of flavour release profiles into the breath from orodispersible tablets of varying diameter or thickness, effervescent tablets having different formulations, or the effect seen of incorporating flavours in the dispersed phase of emulsified syrups. Similarly, the persistence in the breath of flavour used to mask off-notes may be readily determined; the value of such information lies in the general observation from APCI-MS data that persistence in the breath can vary greatly between individual flavour compounds.

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The speed of data collection also offers substantial advantages. Several technological solutions exist to mask unpleasant flavour impressions, most based on preventing in-mouth drug dissolution, but these are system-specific and expensive in human and material resources. For this reason, pharmaceutical formulators usually select flavouring as the initial masking approach. For instance, numerous examples of bitterness inhibitors (mostly non-volatile) are documented [3–6]. Typical flavour orientation is used to improve the taste and odour of antibiotics, antihistamines, barbiturates, sulfa drugs, salicylic acid derivatives and vitamin preparations [7]. However, the usual approach in working towards an acceptable flavour experience when a drug is taken orally is essentially one of trial and error based on the experience of formulation experts with continuous feedback from sensory analysis. This is necessarily costly in the human resources required.

1.1. Flavour fundamentals

The release of flavour molecules from food during eating or any medium taken orally is complex. It is difficult to generalise about changes that occur in the mouth owing to the enormous range of food types [8]. Factors such as the shearing and mixing action of chewing, dissolution in or dilution by saliva, thermal melting or hydration, combine to provide a dynamic pattern of partitioning between the various solid/liquid phases and the breath. Stimuli arising from interactions of volatile molecules in the breath with sensors in the nose (olfactory epithelium and trigeminal) are combined on a neural level with stimuli by non-volatiles in the mouth (taste receptors and trigeminal), and with textural/mouthfeel and temperature sensations to create an overall impression we describe as flavour [9]. While the pattern of stimulation varies with food type, the contribution to flavour perception from volatile molecules in the breath is usually the dominant factor, as demonstrated by the commonly experienced difficulty in differentiating between flavours when the nose is blocked.

A further advantage of monitoring breath volatiles using APCI-MS therefore lies in its objectivity. For example, a study by sensory evaluation of liquid antacids found a relation between sample ageing and reduced flavour perception and with greater perceived viscosity [10]. APCI-MS could be used in this case to ascertain the extent to which the reduction of perceived flavour with ageing was due to lower levels of volatiles released into the breath, as opposed to a neural processing effect dependent on perceived viscosity.

1.2. A new technology

APCI-MS has been developed for near universal breath volatile analysis by Prof. A. Taylor and Dr R. Linforth at the University of Nottingham in a joint project with Firmenich S.A., a leading manufacturer of flavours and fragrances based in Geneva. It has led probably more than any other approach towards a better understanding of the behaviour of flavour volatiles in the mouth and nose, and how this affects flavour perception. Firmenich currently operates two such machines (one modified model Platform II, one model ZMD with fitted MS-Nose®, both from Micromass, Manchester, UK) under the trademark AFFIRM®, an acronym of Analysis of Flavours and Fragrances In Real tiMe. The key features of AFFIRM® technology are:

- the capability to monitor flavour volatiles quantitatively in the breath on a breath-by-breath basis over the range of concentrations typically released during eating and drinking, or continuously in the headspace (the gas phase above any solid or liquid phase, including tablets and syrups, incorporating volatile molecules); and
- the rapidity of data collection.

This review contains an overview of the development and principles of operation of the technique, a summary of its more important findings and applications to date, and proposals of how it may be used further to advantage in pharmaceutical applications.

2. Real time breath analysis

2.1. Background

The most satisfactory method of analysing breath volatiles in the early 1990s was by using GC-electron impact MS analysis, which required a concentration stage of trapping, either cryogenically or on an absorbent polymer before injection into the GC column [11–13], owing to the low concentrations of volatiles typically found in breath. Disadvantages of this approach were high labour intensity, time-averaged results and in the case of polymer traps, selective absorption of volatiles. One important finding that was apparent from these methods was that actual concentrations of volatiles in the nose and mouth changed with time.

Direct sample introduction into a mass spectrometer had long been considered an attractive possibility as the high sensitivity of the technique would both allow volatile analysis on a breath-by-breath basis and dispense with the need for pre-concentration. Breath-by-breath profiles were thought to be very similar to those sensed by the olfactory epithelia and were expected to be different from the volatile composition of food and headspace profiles owing to the physical changes undergone by food during eating [14]. However, a satisfactory method to introduce breath into a mass spectrometer remained elusive for some time, as the performance of conventional electron impact mass spectrometers is severely reduced by both oxygen and water. One solution to this problem was to fit a polymer membrane filter to a mass spectrometer inlet to exclude air while

allowing volatiles to diffuse into the source [15]. However the membrane was selective and introduced a time lag, preventing both universal application for flavour volatile analysis and breath-by-breath time resolution.

2.2. Atmospheric pressure chemical ionisation mass spectrometry

A major advantage offered by APCI-MS is that volatile signals are not poisoned by oxygen or water, which allows breath to be introduced directly into the MS source. In fact, water is essential because it is the reagent ion when used for breath analysis in positive ionisation mode. The charge is borne by water cluster ions of the general form $H_3O^+.(H_2O)_n$. As volatile organic compounds used in flavouring invariably have proton affinities greater than those of the water clusters, transfer of single protons from water clusters to the volatile molecules is energetically favourable. In addition, APCI is a 'soft' ionisation technique, meaning that there is normally insufficient energy to fragment a molecule following ionisation as occurs in electron impact MS. The main, and usually the only significant ion formed from a vast majority of flavour volatiles, M, is the protonated intact molecule, MH^+. Exceptions are alcohols, which dehydrate, as do aldehydes to a lesser extent. Volatiles can thus be monitored according to their molecular weight and a number of volatiles can be monitored simultaneously. The temporal separation of a GC column is thus eliminated. Although the method is rapid and simple to use, one drawback that is its major disadvantage, is that molecules which generate identical mass/charge ions cannot generally be differentiated.

An interface for direct breath sampling into an APCI-MS source was demonstrated as early as 1983 [16]. Among the disadvantages of that version was the need for the subject to regulate the pressure in the inlet chamber by the strength of exhalation, which rendered it somewhat impractical for general use. APCI proved to be a difficult process to control for gas phase volatile analysis and in the subsequent decade a number of attempts were made to apply it to achieve quantitative gas phase analysis, with limited success. The key developments during this period, and a detailed account of the principles and scope of APCI-MS as currently applied to breath analysis are presented elsewhere [17]. A brief summary of operational aspects is given below.

An alternative soft ionisation MS technique, proton transfer reaction (PTR)-MS, in which reagent ions are formed before mixing with the sample of air containing volatiles, has been applied to a range of trace volatile analysis applications, including that from foods [18]. However, as the published data contains little information on flavour release in vivo, it is difficult to evaluate the technique for breath-by-breath analysis.

2.3. Real time nosespace analysis

In the present APCI-MS system for analysis of breath from the nose (nosespace), developed in 1996, well over 90% of flavour volatiles can be monitored quantitatively on a breath-by-breath basis. A schematic diagram of nosespace sampling is shown in Fig. 1. A detachable stainless steel tube is inserted loosely in one nostril such that the air sampled is that which has passed over the olfactory epithelia. The nosepiece tube is adjustable horizontally and vertically allowing greater comfort than might appear at first sight. The subject is asked to adopt a relaxed, regular breathing pattern. Samples are put into the mouth at predetermined intervals, chewed and swallowed normally. The mouth is kept closed apart from when taking in samples to ensure that all expired air passes through the nose. A small part of the breath is drawn into the MS source via an interface of particular design [19,20] incorporating a venturi, a passive pumping device in which constant suction is provided by a nitrogen carrier flow of 10 l/min. The sampling rate into the MS is adjustable and is typically set in the range 5–50 ml/min, depending on the volatile load.

The transfer line through which the breath sample passes is of fused silica of internal diameter 0.53 mm, which is deactivated to minimise volatile adsorption. It is sheathed in an electrically heated element to prevent water condensation. Nosespace analyses are run in selected ion mode, which allows the cone voltage to be set for maximum sensitivity for each of a number of compounds monitored simultaneously. While the Masslynx software allows 32 channels to be monitored simultaneously as a function of time, there is a loss of sensitivity with an increasing number of channels and usually up to 8–10 are followed. As volatile identification is by mass alone, it is necessary to know in advance which volatiles are present. If not, an initial qualitative determination is required, usually by trapping headspace volatiles followed by GC-MS analysis, to determine not only the molecules present but also whether the masses to be monitored are unique to the compounds of interest.

Reproducibility between replicates of samples eaten by the same person is usually fairly good and normally no special training is needed beyond an initial familiarisation. This is not true however for liquid samples, owing to the short timescale of the drinking process, the rapidity of volatile release, and if samples are drunk from a cup, the need to remove the nosepiece from the nostril to sip each replicate. Good reproducibility may still be achieved following a program of panellist training.

Calibration is achieved by injecting solutions of pure volatile in an inert solvent (i.e. one having a proton affinity lower than that of the water molecule clusters, such as cyclohexane) into the carrier gas flow. As the volumes injected are small and the flow rate of the carrier gas is fast, the solvent and liquid volatile are vaporised immediately. A large majority of flavour volatiles show linear concentra-

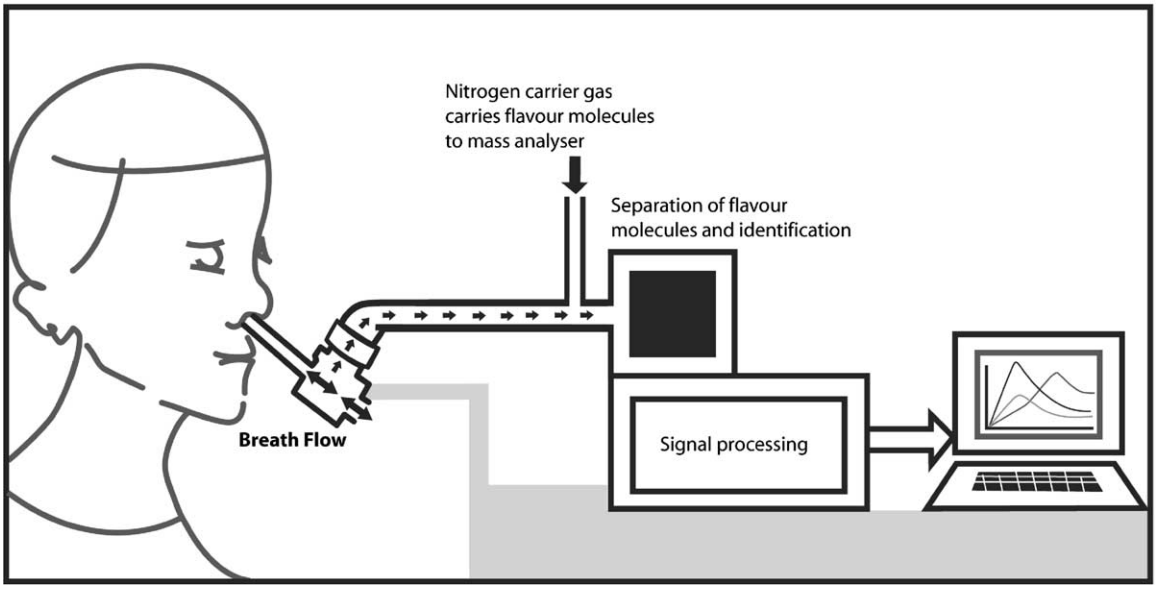

Fig. 1. Schematic diagram of AFFIRM®ative nosespace analysis.

tion/response curves over a range typically from a few ppb to 1 or 2 ppm, which coincides with the concentration range of volatiles most commonly found in the breath during eating. Detection limits vary between flavour volatiles; a value of 1 ppb is typical. This sensitivity allows measurement of some 80% of flavour volatiles at their threshold of perception [21]. Some volatiles, notably many of those containing sulfur, are organoleptically active at concentrations below the detection limit of the equipment and so cannot be monitored in breath.

As the equipment has a rather high nitrogen consumption, use of a nitrogen generator operating from a compressed air supply is recommended; this removes the need for frequent, and in the long term expensive, changes of nitrogen bottles. A zero air generator may be installed downstream of the nitrogen generator to remove any residual contaminants and allow very low and stable baselines throughout the useful working mass range.

The large volumes of data recorded make efficient processing procedures imperative. Software packages have been developed at the University of Nottingham and at Firmenich to process the raw data and present it in formats of the greatest practicality with maximum flexibility in a minimum of time.

2.4. Time dependence of volatile release

Consideration of the time dimension of flavour volatile release into the breath during eating (flavour dynamics) has been shown to be no less important than quantification of concentrations. Volatile concentrations vary from breath to breath depending on the interactions between physiological factors (e.g. manner and rate of chewing, breathing rate, saliva flow rate) and properties of the food (e.g. composition, structure, texture). However, a further source of variation arises from physicochemical properties of the volatile molecules, which may have a major effect. Fig. 2 shows the different temporal release profiles into the breath of five volatiles commonly used in fruit flavours from gelatin/pectin gels made to a standard recipe and eaten by the same person. Each curve is the mean of five replicates, normalised to the maximum intensity. Mean times to reach the maximum concentration, T_{max}, vary from 0.15 min for limonene to more than 0.5 min for hexenol. It is clear that the relative composition of volatiles in the breath in this example change dramatically from breath to breath; only a technique having the time resolution capability of APCI-MS can demonstrate this objectively. Such differential temporal release of volatiles from a common matrix is a general phenomenon and clearly has profound implications for flavour perception.

Some progress in elucidating those parameters of volatile molecules which are most important in determining release properties has been made using an empirical quantitative structure–property relationship (QSPR) approach. At the least complex level, a QSPR study of persistence in the breath has been made of a test set of 41 flavour volatiles drunk individually in dilute aqueous solutions [22]; volatile persistence was shown to vary greatly among the volatile test set. Hydrophobicity and vapour pressure of the volatiles were found to be major components, although not the only components, of a QSPR model, which was then shown to have some predictive capacity.

3. Applications of nosespace analysis

3.1. Nosespace analysis and perception

One of the most rewarding applications of nosespace

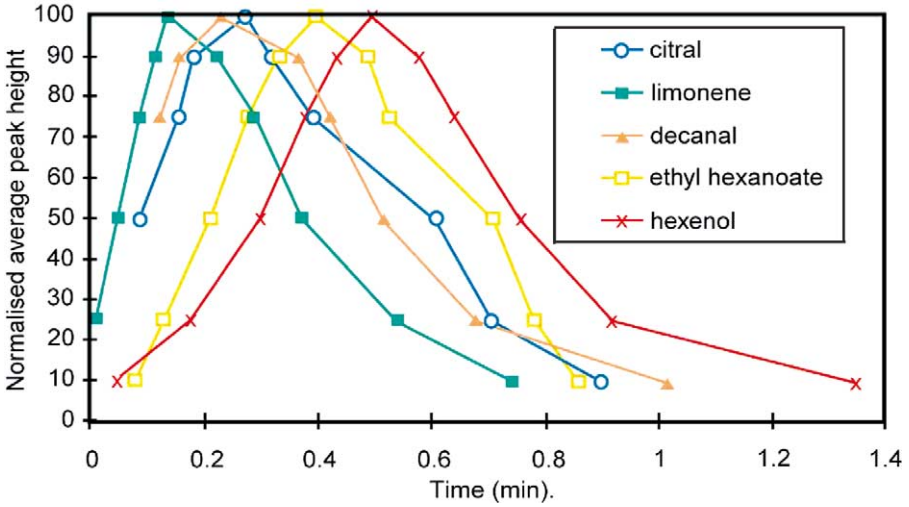

Fig. 2. Volatile release profiles in-nose from gelatin/pectin gels normalised to maximum intensities (reprinted with permission from Harvey et al. [29]. Copyright 2000 American Chemical Society).

analysis is to conduct simultaneous sensory evaluation, i.e. panellists are asked to record aspects of the flavour they are perceiving while an objective measure of the flavour dynamics is recorded. In this way a better understanding may be obtained of those factors most affecting perception. Any sensory test may in principle be conducted; at Firmenich, Fizz® sensory acquisition software (version 2.0, Biosystèmes, Dijon, France) is used.

Time–intensity sensory evaluation lends itself particularly to this approach; for example, parallel sensory and instrumental time–intensity curves may be recorded of chewing gums of different gum base composition or flavour formulation. Trained sensory panellists are asked to move a cursor along a linear scale using a mouse (Firmenich) or move a lever (Nottingham) according to their perception of some flavour descriptor, but are prevented from seeing the nosespace data generated to avoid any feedback which may influence perceived ratings. In the simplest case a panel may be asked to estimate overall flavour intensity. The sensory and instrumental data are processed in parallel by panellist and correlations are sought between sensory and instrumental parameters describing the curves across all panellists. If more than one flavour volatile is present, though, care is needed in data interpretation.

Such an approach was applied at the University of Nottingham using gelatin gels containing a fixed dosage of a single flavour volatile but varying gelatin content [23]. The maximum perceived flavour intensity correlated well across a panel of 11 people with the initial gradient of the nosespace release profile but not with the maximum concentration recorded in the nose, providing evidence that the rate of change of volatile concentration in the nose is more important than the absolute flavour dosage in determining the perceived flavour impact. Another factor discovered from further experiments with gelatin gels was a variation in the relation between T_{max} times recorded instrumentally and those recorded sensorially by the panellists [24]. When gels of low gelatin content were eaten, consumption times were short and sensory T_{max} times were mostly greater than the instrumental T_{max} times. A delay in aroma sensations reaching maximum intensity has been attributed to the temporal integration of the stimulus [25], and such a temporal integration lag was proposed throughout the time of eating the gelatin gels, particularly in the early phases [24]. In contrast, when harder gels requiring longer eating times (>45 s) were consumed, sensory T_{max} times were mostly shorter than instrumental T_{max} times as sensory adaptation became an important factor before the gels were swallowed. These results are directly applicable in the pharmaceutical field, where flavoured gelatin gums have been manufactured to improve the palatability of midazolam [26] and paracetamol [27] for children.

A further important factor in the flavour perception of beverages is viscosity. The effect of viscosity on both flavour perception and nosespace volatile concentrations has been investigated in parallel [28]. Trained sensory panellists were presented with aqueous solutions containing hydroxypropyl methylcellulose (HPMC) thickener at concentrations of up to 2% and fixed levels of sucrose and strawberry flavour. The mean perceived intensity of both flavour and sweetness was constant at HPMC levels below its C^* value (the concentration at which the biopolymer chains start to entangle) and dropped approximately linearly with HPMC concentration above C^*. However, nosespace volatile levels of ethyl butyrate from the strawberry flavour were unexpectedly constant over the whole range of HPMC concentrations, both below and above C^*. It was then shown how levels of flavour volatile and sugar could be adjusted to compensate for the effect of viscosity on perceived flavour.

Findings such as described above have opened up new potential for the design of food flavouring systems having desired perceived flavour profiles when eaten in particular matrices [29]. While nosespace analysis does not, and cannot, take the place of sensory analysis, the speed of data

collection and objectivity provide a valuable complement to sensory work. In many instances, nosespace analysis may be used as an initial screening stage before products are presented to sensory panels, allowing large savings of human resources.

This is particularly valuable in the pharmaceutical field where evaluation of the flavour of a drug dosage form by a large number of people may be impractical owing to internal (e.g. drug availability in a preformulation stage) or external (e.g. setup of a clinical study) limitations. Using APCI-MS, the formulator would have rapid access to data quantifying the effect of ingredients and process conditions on flavour release, from which trends in factors affecting patient acceptability might be inferred at an early stage. A major potential area of application lies in quality control in the pharmaceutical industry, where investment in sensory testing may be substantially reduced, in applications such as has been reported, for example, of mint flavoured antacid suspensions [30].

3.2. Analysis of non-volatiles released in the mouth

An instrumental technique has been developed which is complementary to nosespace analysis and which allows the release of non-volatiles in the mouth to be quantified [31,32] with time during eating. Two alternative methods of sampling saliva have been successfully applied; in one method, samples are taken by cotton swabs at predetermined time intervals and in the other sampling is continuous on adsorbent ribbon passed through the mouth at constant speed. In both methods, saliva is solvent extracted from the sampling medium and injected into the MS. In this way, the contribution to flavour perception of non-volatile release in the mouth as a function of time can be considered. Although flavour volatiles are usually responsible for the major part of overall flavour perception, release of sugar from mint flavoured chewing gum was shown to determine the form of the time–intensity profile of perceived flavour recorded by a trained sensory panel rather than the nosespace profile of menthone, monitored as a marker of the mint flavour. Related methods for monitoring acids and electrolytes have also been developed [33]. Information of this kind could be of use, for example, in understanding the preference between formulations of medicated chewing gum, as reported from patient acceptability of two nicotine chewing gums [34].

3.3. Product development

Nosespace analysis is routinely used at Firmenich to investigate the effect on volatile release profiles of factors such as the matrix composition of flavour encapsulants [29] or fat levels [35], both of which can have dramatic effects. Fig. 3 shows nosespace release profiles of menthol from chewing gum batches containing liquid peppermint oil or the equivalent dosage of peppermint oil encapsulated in one of the Flexarome® range of products from Firmenich. The flavour burst in the first few minutes from the chewing gum sample containing the Flexarome® product is due to the physical protection provided by the carbohydrate matrix, which prevents the flavour volatiles partitioning into the gum base until the Flexarome® pieces are crushed during chewing or dissolve in saliva. When liquid peppermint oil is added to chewing gum, most of the flavour molecules reside preferentially in the gum base owing to the high hydrophobicity of the volatiles contained in the mint flavour.

4. Headspace analysis

4.1. Static headspace

While AFFIRM® technology was developed primarily for dynamic breath analysis, it can in principle be readily adapted to monitor volatiles in any gas phase. A number of transfer lines of different lengths and electrically heated at temperatures up to 150 °C are quick to fit and allow great sampling flexibility. Many applications of headspace analysis have been developed, mostly when the capacity to monitor the kinetics of gas phase volatile evolution is of interest.

The advantages of rapidity of measurement and sensitivity when compared with other headspace techniques also make it attractive, and in some instances the method of preference, for quantifying equilibrium headspace concentrations. One simple application is the determination of air–liquid partition coefficients of compounds of low volatility from series of related systems, when headspace concentrations may be too low for reliable quantification by alternative methods.

The most reproducible results are obtained using an open split with the venturi set to sample the extracted headspace into the MS at a fairly low flow rate. Alternatively, many static headspace measurements may be carried out rapidly by manually changing the appropriate connections to the MS interface. Signal equilibration times when measuring headspace at equilibrium are normally short, owing to the stability of baselines and of the sampling rate into the MS. A number of volatiles may be determined simultaneously and calibration is achieved using external standard solutions as for nosespace analysis. Connection to an automatic headspace sampler is straightforward, if required.

A series of air–liquid partition coefficient measurements were recently conducted to develop and test a predictive QSPR model, using molecular parameters of volatiles, of headspace concentrations above sucrose solutions [36]. Such data is of fundamental value in understanding the dynamic aspect of flavour release as described below. A review of the partitioning of flavour volatiles between phases describes basic principles, an overview of measure-

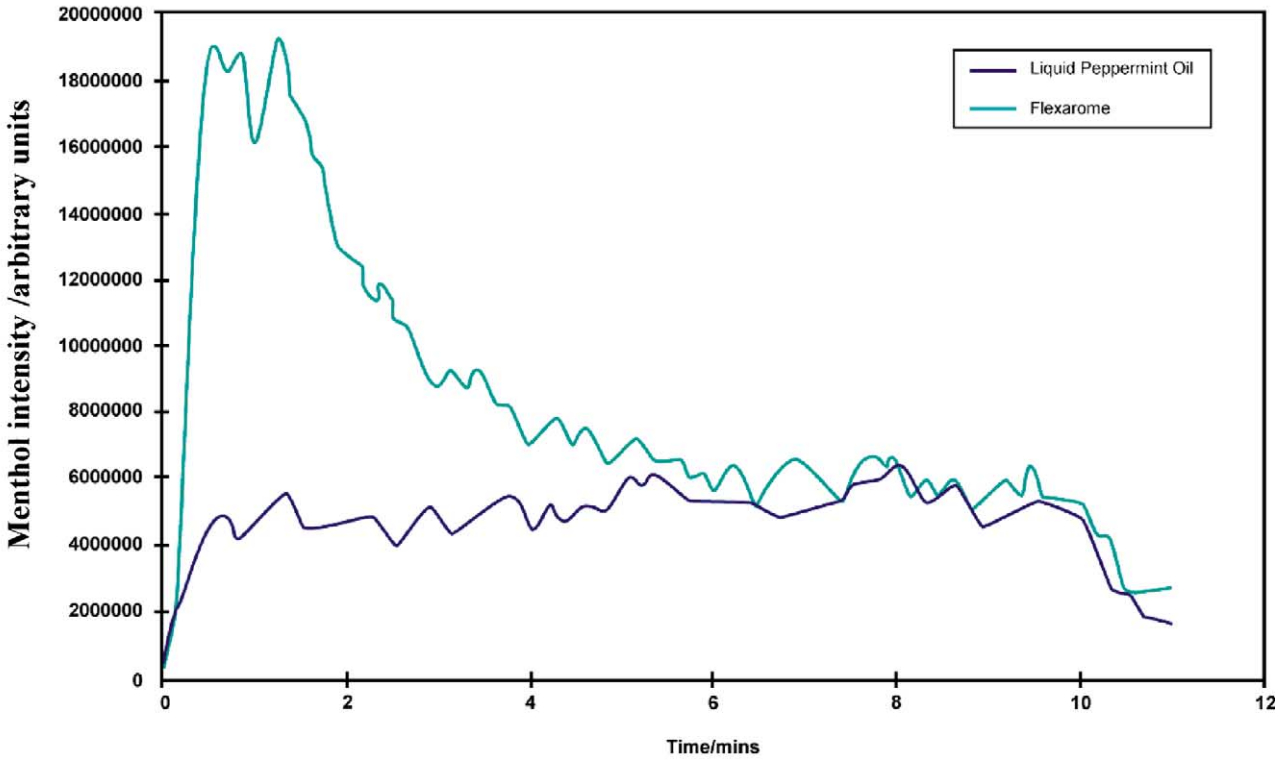

Fig. 3. Release profiles of menthol from chewing gum containing an equivalent flavour dosage in liquid form or encapsulated in a Flexarome® product.

ment techniques and some applications to food systems [37].

4.2. Real time headspace measurement

In systems where change in volatile headspace concentrations is rapid, the temporal resolution is limited only by the design of the MS and its software. In selected ion mode, measurement of headspace concentrations is possible at intervals as short as 10 ms if required. At the other extreme, kinetic profiles may be monitored for periods of 24 h or more without operator intervention when the change in headspace concentrations is slow.

Generation of Maillard reaction products from heated skimmed milk powder have been monitored as a function of temperature [38]; this application has much potential for following reaction pathways and analysing reaction kinetics in Maillard processes. Other real time kinetic analyses of volatiles in the headspace (unpublished results) have been made of:

- volatile release to the headspace as flavoured tablets of different matrix composition or different flavour encapsulation media dissolve in water under agitation;
- flavour loss when food is cooked in a benchtop fan oven or microwave oven; and
- differential release of volatile components of perfumes in, e.g. fine fragrance from skin, shampoo from hair, fabric conditioner from textiles during drying, etc.

In the pharmaceutical field, a number of applications in addition to monitoring the flavour development of solid and liquid dosage forms may be envisaged, with the reservation that the analyte must have a proton affinity greater than that of the water molecule clusters. A database of evaluated proton affinities of approximately 1700 molecules has been compiled [39], from which proton affinities of some other molecules of pharmaceutical interest may be estimated by structural analogy. Potential pharmaceutical uses include kinetic analyses of:

- release of volatile drugs such as analgesic aerosols used in Chinese traditional medicine from volatile oils such as *Piper longum*, *Santalum album*, *Dryobalanops aromatica*, *Asarum sieboldi* and *Alpinia officinarum* [40];
- the evolution of encapsulated perfumes [41] released from pharmaceutical creams and ointments; and
- the effects of pulmonary first pass of volatile or gaseous compounds administered intravenously on the calculation of apparent volumes of distribution and pulmonary clearances.

4.3. Validation of mathematical models

While the kinetics of volatile release can be readily measured and compared from solid or liquid systems of varying formulation or under different conditions using APCI-MS, the capacity to monitor the kinetics of volatile release into the headspace systematically in model systems

can also provide a valuable tool in helping to elucidate the most important factors controlling volatile release. The development and validation of mathematical models of flavour release with greater predictive power can thus be greatly facilitated using the technique, which should then enable reductions in the resources required for product development.

Mechanisms of flavour release from foods are in general not well understood. A number of theoretical models of volatile release from foods have been proposed, depending on the food type and primary release process (e.g. melting, solubilisation) [14,42], but these generally lack experimental validation which it is now possible to provide using APCI-MS. The different approaches to modelling of flavour release have been reviewed and assessed [43]. A summary of predictive models which have been validated by APCI-MS is presented below.

Progress has been made in understanding the dynamics of volatile release from as relatively simple a system as an aqueous solution, where a mechanistic model has been developed and validated using APCI-MS experimental data [44]. The headspace over aqueous solutions of selected volatiles initially at equilibrium was diluted with air under laminar flow conditions, a process which can be related to opening a sealed beverage container. Volatile concentrations in the headspace changed with time, but at different rates depending on the rate of replenishment from the aqueous phase. The equilibrium air/water partition coefficient, K_{aw}, was shown to exert a major effect on dynamic volatile release and was the rate determining factor for volatiles having K_{aw} values $<10^{-3}$, whereas mass transport in the gas phase became significant for volatiles of K_{aw} values $>10^{-3}$. This approach was subsequently extended when a dimensionless parameter depending on the gas flow rate, and on the mass transfer coefficient and surface area between the stirred aqueous phase and a gas phase, was identified as driving the physical mechanism of flavour evolution [45]. A gas–liquid interfacial mass transfer cell has been designed to be coupled to an APCI-MS to investigate systematically the effect on flavour volatile transfer kinetics from a flowing liquid to the gas phase of factors such as viscosity, binding to macromolecules and properties of emulsions [46].

It has been generally observed that the composition of volatiles in the headspace of any food differs, often substantially, from that measured in the nosespace when the food is eaten, and that nosespace volatile concentrations are usually much lower than those found in the corresponding headspace. A recent series of parallel headspace and nosespace measurements identified the most important factors contributing to these differences [47]. It was deduced that the dominance of the mass transfer coefficient between aqueous and gas phases in vitro [45] under conditions of air dilution was applicable in vivo; nosespace/headspace concentration ratios were compound specific and could be as large as two or even three orders of magnitude. Further reductions in concentrations of volatiles released during eating when exhaled retronasally were shown to result from gas phase dilution in the upper airway and absorption by the nasal epithelia. In addition, measured volatile concentrations in nosespace were markedly lower than in mouthspace (exhaling through the mouth). Incorporation of volatiles in emulsions was shown both to increase the nosespace/headspace volatile concentration ratio and to enhance the stability of headspace volatile concentrations with time during gas phase dilution [48] when compared with release from water.

5. Conclusions

AFFIRM® is a versatile tool well-suited for the rapid, quantitative analyses of breath or headspace in real time with high sensitivity. The technique is still fairly new and there remains much potential for the investigation of the kinetics of flavour volatile release from a wide range of edible systems, in addition to the significant progress already made. Major areas of application are:

- Objective measurement and comparison of nosespace or headspace volatile release profiles from flavours in different media or encapsulated in different matrices, which may make major contributions to product development.
- Objective screening of flavour performance before sensory testing, which can generate large savings in human resources, an important factor when drug tasting is considered.
- With simultaneous sensory evaluation, identification of factors most affecting flavour perception.
- Development of products with required flavour perception characteristics.
- Development and validation of mathematical models of volatile release leading to increased predictive capability to facilitate product development.

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