15 Advanced Instrumental Analysis and Electronic Noses

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15.1 Introduction

Since the development of suitable gas chromatographic methods in the 1960s, researchers have been able to identify thousands of volatile compounds in foods, essential oils and fragrances. High-resolution gas chromatography (GC) combined with mass spectrometry (MS) became the key technique used for quantification and identification of flavour compounds. Individual flavours were found to be complex mixtures containing hundreds of compounds in concentrations ranging from percent to trace (nanograms per kilogram) levels. Additional information is obtained by smelling the GC effluent after its separation. This technique is called GC-snifing or GC-olfactometry (GC-O). To determine the relative sensorial importance of a volatile compound to the overall flavour, odour activity values are calculated by relating the measured concentration of a compound to its odour threshold. Since this does not work with unidentified and trace compounds, other methods using GC-O for the determination of the specific contribution of gas chromatographically separated compounds have emerged: aroma extract dilution analysis (AEDA), combined hedonic aroma response measurements (CHARM) and OSME (a time-intensity rating method) [1, 2]. These sniffing techniques clearly demonstrated that of all these volatile components only a few contribute to the characteristic odour and only in some cases “character-impact compounds” could be found. Therefore, the comprehensive identification of all substances is no longer the main goal. The aim of modern flavour research is now focused on those volatile constituents which either independently or in combination produce a characteristic aroma response. This forced flavour chemists to develop new advanced techniques, both for isolation and concentration as well as for separation and identification.

Besides classical headspace analysis, simultaneous distillation-extraction and solvent extraction, new sampling and enrichment developments include solvent-assisted flavour evaporation (SAFE) [3] and sorptive techniques like SPME solid-phase microextraction (SPME) [4,5] and stir-bar sorptive extraction (SBSE) [6], which are treated in a dedicated chapter in this book. This contribution will deal with advanced developments of GC techniques for improvement of separation and identification (classical multidimensional GC, or
MDGC, and comprehensive two-dimensional GC, or GC×GC), faster separation techniques (fast GC), fast methods for quality assessment or process control in the flavour area ("electronic noses" and fingerprinting MS) and on-line time-resolved methods for analysis of volatile organic compounds (VOCs) such as proton-transfer reaction MS (PTR-MS) and resonance-enhanced multi-photon ionisation coupled with time-of-flight MS (REMPI-TOFMS). The scope of this contribution does not allow for lengthy discussions on all available techniques; therefore, only a selection of developments will be described.

15.2 Multidimensional Gas Chromatography

Single-column gas chromatographic analysis has become the standard approach for separation of volatile and semivolatile constituents in numerous applications; however, this does not necessarily provide the best analytical result in terms of unique separation and identification. There is considerable opportunity for peak overlaps, both on a statistical basis and also on the basis of observed separations achieved for real samples [7-9].

15.2.1 Classical Multidimensional Gas Chromatography

In order to expand the analytical separation, chromatographers have developed a range of solutions based on more than one separation space. Termed MDGC, it consists of an arrangement of two or more columns where distinctive segments of effluent from the first column are fed into one or more secondary columns (Fig. 15.1).

The entire procedure is enabled by the presence of a specific transfer device between the two columns. Cortes [10] and Bertsch [11, 12] have presented a comprehensive discussion of conventional MDGC technology and their contributions are recommended to readers who wish to have a more detailed outline of MDGC and its applications.

In conventional two-dimensional GC (Fig. 15.1a), discrete fractions of effluent (heart cut) are diverted into a secondary column, generally with a different polarity. This arrangement has the disadvantage that components from different cuts may intermingle in the second column and thus can no longer be correlated. Application of parallel traps is one possibility (Fig. 15.1b) to solve this problem. Of course, this is achieved at the expense of increasing the total analysis time, since each fraction delivered to the trap must be individually analysed in a conventional GC procedure. This disadvantage can be circumvented by using the highly complex arrangement shown in Fig. 15.1c, where each cut is directed into a separate column.

The mechanism by which effluent is switched from the first to the second column is critical. Column flows are diverted basically by using valves or valve-
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Fig. 15.1 Basic arrangements in multidimensional gas chromatography (MDGC): a conventional, b multitrap, c multicolumn

less Deans-type switching devices. Accurate descriptions of the most important MDGC interfaces have been reported in the literature [12]. A truly versatile arrangement is presented in Fig. 15.2 by way of example.

It consists of a dual oven with a valveless Deans-type interface (Live-T switching device) between the columns, an intermediate effluent monitor detector and facilities for flow reversal. Additionally, a device (total transfer) to focus and reinject the desired effluent fraction after the first column allows the combination of a high-capacity precolumn (packed or thick-film wide bore) with a high-resolution capillary or chiral column. Trapping a heart cut before introduction into the second dimension makes the second separation independent of the first. Depending on the application, the instrument incorporates inlets for liquid injection, dynamic headspace enrichment, thermal desorption and facilities for SPME, SBSE and headspace sorptive extraction (HSSE). After suitable enrichment and initial separation on a high-capacity column, appropriate heart cuts are transferred to the main column. After final separation, the substances of interest can be directed via a second Live switching device to an MS, isotope
ratio MS (IRMS), $^{14}$C or Fourier transform IR detector or into individual traps. Accumulation of sufficient material (preparative scale isolation) for further characterisation by off-line instruments such as an NMR spectrometer can be achieved by collection of selected heart cuts from the second column in separate traps after a certain number of GC enrichment cycles [13,14].

Apart from petrochemical and environmental applications [12], classical MDGC was/is used in the flavour field mainly for enrichment and identification of odorous trace compounds in complex mixtures, or for authenticity evaluation by chiral separation or isotopic ratio determination. In Table 15.1 some typical applications are given.

In order to illustrate the potential of MDGC when dealing with complex mixtures, an application to determine off-flavour compounds in defective coffee beans is given by way of example (Fig. 15.3)

As can be seen, MDGC is a targeted analysis applicable to a specific application. Heart cutting can be effectively applied to a small number of regions of interest in the chromatogram. Transferring them to a second column gives enhanced resolution of the heart-cut zones owing to the different column selectivity. Care should be taken that compounds from one cut do not interfere with the separation of another cut. With respect to the maximum numbers of cuts achievable in a single MDGC run, this is dependent on the sample type and on the analytical conditions. A practical implementation of a large number of heart cuts in order to completely separate a complex mixture in a single run is just not possible. Nevertheless, heart-cutting MDGC may be considered for target applications the most appropriate analytical choice.

Fig. 15.2 A two-dimensional GC (GCxGC) system. D1 intermediate effluent monitor detector, column 1 high-capacity precolumn, column 2 high-resolution capillary or chiral column.
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Fig. 15.3 Dynamic headspace MDGC analysis of three coffee beans. a Chromatogram of precolumn showing heart cuts at retention of peasy-like odours detected by sniffing the eluent from the precolumn. b Heart cut from normal beans on the main column. c Heart cut from defective beans on the main column [14]

If the speed of the secondary separation is high enough to separate a cut from the first separation while the next cut is being collected, the complete two-dimensional chromatogram could be constructed. A new type of instrumentation was developed to accomplish this goal. The technique, called comprehensive GCxGC, was introduced in 1991 by Liu and Phillips [30]. It expands the MDGC method into a generally usable format that does not rely on targeting specific zones of a first-dimension analysis.

15.2.2 Comprehensive Two-Dimensional Gas Chromatography

Comprehensive GCxGC uses the whole two-dimensional separation space to generate resolution, provided that the individual dimensions are orthogonal. GCxGC consists of two chromatography columns, serially coupled, with a modulation mechanism at their junction. A typical column set is composed of a standard low-polarity column (first dimension), with typical dimensions of 25 m x 0.25-mm internal column diameter x 0.25-μm film thickness, coupled to a much shorter and more polar second column (second dimension) (or a column providing a separation mechanism capable of further differentiating target sample components), with dimensions of 1 m x 0.1-mm internal column
Table 15.1 Selected applications of multidimensional gas chromatography in flavour research

<table>
<thead>
<tr>
<th>Application</th>
<th>References</th>
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<tbody>
<tr>
<td>Enrichment of trace compounds from interfering complex mixtures</td>
<td>[15, 16]</td>
</tr>
<tr>
<td>Identification of odour-active undecaenes in fruits and vegetables</td>
<td>14, 17, 18</td>
</tr>
<tr>
<td>Identification of a peasy off-flavour in coffee beans</td>
<td>[19]</td>
</tr>
<tr>
<td>Identification of musty/earthy off-flavours in wheat grains</td>
<td>[14]</td>
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<tr>
<td>Identification of a rotten off-flavour in car mats</td>
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<tr>
<td>Enantioresolution of lactones for authenticity control of fruit products</td>
<td>[20-24]</td>
</tr>
<tr>
<td>Enantioresolution of terpenes for authenticity control in essential oils</td>
<td>[24-26]</td>
</tr>
<tr>
<td>Enantioresolution of nor-carotenoids</td>
<td>[27]</td>
</tr>
<tr>
<td>13C/12C isotope ratio determination for authenticity control of flavours</td>
<td>[23, 24, 28]</td>
</tr>
<tr>
<td>Preparative-scale enrichment</td>
<td>[13, 29]</td>
</tr>
<tr>
<td>Isolation of terpenes from essential oils</td>
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The modulator interfaces the two coupled columns, and is responsible for the quantitative transfer and compression of all solutes, or a representative fraction thereof, from column 1 to column 2. To accumulate the analyte in narrow bands in the modulator, either elevated temperature (the thermal sweeper) to accelerate the solute into a narrow band or cryogenic means to retard the analyte and cause on-column trapping is used [31]. The operation of a dual-jet cryogenic system is explained in Fig. 15.4.

The resulting very sharp peaks are then released onto the short fast column 2. The modulator actually collects eluent from column 1 every few seconds (generally 2–9 s), and so an individual chromatographic peak is actually sliced into many fragments. Figure 15.5 demonstrates how two overlapping peaks are effectively deconvoluted into two interleaved series of pulses.

Each fragment is focussed and pulsed to column 2 for fast analysis. Because modulation is a mass-conservative process, the peak height increases to accommodate the reduction in peak width; thus, greater analytical sensitivity is obtained. Provided that column 2 can resolve the substances focussed by the
Fig. 15.4 A dual-jet cryogenic modulator. a Right-hand-side jet traps analytes eluted from the first column; b right-hand-side jet switched off, cold spot heats up rapidly and analyte pulse is released into the second column; simultaneously, left-hand-side jet switched on to prevent leakage of first-column material; c next modulation cycle is started (adapted from [32]).

Fig. 15.5 Illustration of how two overlapping peaks δ and φ emerging from the first column (a) are resolved in GC×GC after passage to the second column (b) [31]. Reprinted from Marriott, P., Shellie, R., Principles and applications of comprehensive two-dimensional gas chromatography. Trends Anal. Chem. (2002), 21:573–583 with permission from Elsevier.
modulator, compounds which would coelute under conventional single-column GC (Fig. 15.5a) will be separated because of the modulation process, and different pulses can be assigned to different compounds depending upon their retention times (Fig. 15.5b).

The data are generally presented in a two-dimensional plane, which plots the retention time on column 1 (minutes) against the retention time on column 2 (seconds), or a three-dimensional plot where the detector response is also included. In performing a carefully tuned GC×GC experiment, the peak capacity of the overall separation is approximately equal to the product of the peak capacities of the individual separation steps [33]. Thus, the opportunity to characterise mixtures fully is far greater using GC×GC than for both single-column GC and MDGC.

Regardless of the type of analysis, many variables need to be adjusted for optimal performance. It is generally desirable that chromatography in the second column is complete before another aliquot is transferred from the primary column. The need for rapid elution from the second column sets practical limits. In order to get maximum separation performance, each individual first-dimension peak should be modulated into several fractions (in general some five to ten). For that purpose, the ratio of separation speeds between the second and first dimensions must be at least on the order of 50 [30]. Generally, complete elution within a time frame of 2–8 s is required for column 2. With a half peak width of 0.2 s in the second dimension, and the acceptance that at least ten points per peak half width are required to be suitably measured by a chromatographic detector, fast electronics for detection and data collection are needed. In GC×GC to date, detection techniques employed include flame ionisation detection (FID), sulphur chemiluminescence detection (SCD), atomic emission detection (AED), electron capture detection (ECD), nitrogen chemiluminescence detection (NCD) and MS detection (both time-of-flight MS, or TOFMS, and quadrupole MS, or qMS) [34]. In order to conduct GC×GC, the scan speed of the detector is critical: each detector used must be critically evaluated with respect to operational considerations that may limit or affect performance. A possible alternative detection technology, described recently for fast GC, is the surface acoustic wave (SAW) sensor [35].

Several approaches are reported to perform peak quantification in GC×GC. The most common one integrates all individual second-dimension peaks by means of conventional integration algorithms, and then sums all peak areas belonging to one compound. For another method, firstly a so-called base plane is subtracted, and subsequently three-dimensional peak volumes are calculated by means of imaging procedures. Although the peak capacity of GC×GC is high, peak overlapping in two-dimensional separation is very possible, especially for highly complex samples. Chemometric methods, like the generalised rank annihilation method (GRAM), have been used to resolve and quantify severely overlapped GC×GC peaks. Some other methods have also been used, like curve fitting, wavelet analysis [34] and orthogonal projection resolution [36].

The practicability and potential of comprehensive GC×GC coupled to TOFMS (GC×GC-TOFMS) for the analysis of complex mixtures is illustrated in the following application [37].
Figure 15.6 shows the separation achieved for the essential oil of *Coriandrum sativum* using GC×GC. The identity of the compound was elucidated and confirmed primarily from the MS library matches as well as by comparing the first-dimension retention index with reference libraries. GC×GC-TOFMS allowed the identification of 81 compounds, compared with only 41 compounds identified by conventional GC-qMS.

A great advantage of GC×GC is that homologous series of compounds form linear relationships in the two-dimensional separation plane. Figure 15.7 shows the homologous series of compounds identified in *C. sativum* essential oil. This provides another method to confirm compound identity and allows easy discrimination between series of isomers. For example, (E)-2-alkenals and (Z)-2-alkenals exhibit very similar mass spectra but are easily distinguished by GC×GC (Fig. 15.7). In addition, many of the heavier compounds were not present in the mass spectral libraries (and so were often misidentified as lower-mass homologues) but were easily identified using their homologous series.

It is important to appreciate that whilst the GC×GC analysis might not be any faster overall than normal capillary GC, within a similar analysis time, higher sensitivity, greater peak resolution (and hence one could expect greater precision of analysis) and a fingerprint pattern that may contain much subtle information on the chemical class composition of samples, which cannot be achieved in any other way, is obtained.

Selected examples of application areas of GC×GC are presented in Table 15.2. For further details about instrumentation and applications of comprehensive GC×GC, the contributions in [32, 38, 39] should be consulted.
Fig. 15.7 Apex plot showing the homologous series of compounds present in coriander essential oil. The apex of each peak is plotted as the second-dimension retention time against the first-dimension retention time. Reprinted with permission from [37]. Copyright (2005) Wiley

15.3 Fast Gas Chromatography

Conventional GC allows effective separations of complex natural mixtures, but this is frequently achieved at a high cost in time. This becomes a limiting factor, especially for laboratories with a high sample throughput and/or where the need for quick results for the determination of quality and authenticity are required. In recent years there has been increasing interest, within the chromatographic community, towards the development of faster separation methods without considerable loss of resolving power. Various approaches have been theorised and developed with various proposals: shorter column lengths [66], reduced internal column diameter and stationary phase thickness (narrow-bore column) [67, 68], microparticle-packed capillary columns [69, 70], multicapillary columns [71], vacuum-outlet conditions [72], turbulent flow [73] and helically coiled columns [74]. Reviews describing the most important existing high-speed GC methods, also trade-offs and compromises in terms of sensitivity and/or selectivity in combination with MS, have been published [75, 76].

The narrow-bore column approach is a very effective and is the most popular way of increasing analysis speed. Substantial reductions in analysis times are achieved by exploiting two factors: a shorter column length and the application of higher than optimum average linear velocities. Operating under optimum
Table 15.2 Selected applications of comprehensive two-dimensional gas chromatography

| Plant constituents          | Enantiomeric alkaloids [40] |
|                            | Volatiles from germander [41] |
|                            | Tobacco essential oil [44]   |
|                            | Lipids in lanolin [50]       |
|                            | Coriander leaves essential oil [37] |
|                            | *Pistacia vera* essential oil [58] |
|                            | Sandalwood oil [55]          |
|                            | Tea tree and lavender essential oils [60] |
|                            | *Origanum micranthum* essential oil [61] |
|                            | Hop essential oil [65]       |
| Food                       | Flavour compounds in butter [48] |
|                            | Volatiles in strawberry cultivars [54] |
|                            | Roasted coffee bean volatiles [56] |
|                            | Methoxy pyrazines in wine [51] |
|                            | PCBs in milk and cheese [53]  |
|                            | Trace odorants in sour cream [63] |
|                            | Fatty acid composition in foods [62] |
|                            | Citrus essential oil [64]     |
|                            | Yeast cell metabolites [45]   |
| Fragrances                 | Perfume analysis [52]        |
|                            | Allergens in fragrances [59]  |
| Human breath               | Volatile organic compounds [42] |
| Petrochemicals             | Oil spill [43]               |
|                            | Composition [46]             |
|                            | Diesel fuel hydrocarbons [47] |
|                            | Sulphur compounds in crude oil [57] |
| Environmental              | Polychlorinated alkanes in dust [49] |

Experimental conditions, a 10 m × 0.1-mm internal diameter, 0.1-μm film thickness column is characterised approximately by the same resolving power as a 25 m × 0.25-mm internal diameter, 0.25-μm film thickness column [67]. Figure 15.8 shows the chromatograms of conventional GC and fast GC analysis of a lemon oil with the aforementioned columns [77].
As can be seen, 57 components were separated with both methods. The fast GC technique performs the same separation within 9 min, a speed gain of a factor of 5 compared with the conventional method. A lime oil sample, in an application aimed at quality control, was separated satisfactorily in only 90 s on a 5 m x 0.5-mm internal diameter, 0.05-μm film thickness column [78]. Other applications include essential oil analyses [79,80], flavour volatiles in fruits [81], fatty acid composition [82] and pesticides [83].

Fast GC requires instrumentation provided with high split ratio injection systems because of low sample column capacities, increased inlet pressures, rapid oven heating and fast electronics for detection and data collection. Hydrogen is generally used as a carrier gas because of the flatness of its Van Deemter height equivalent to the theoretical plate (HETP)-μ curve, which allows higher linear gas velocities to be applied than the optimum without substantial loss of resolution. Shorter columns, thinner films and smaller internal diameter columns used in fast GC require smaller amounts of sample to be injected to prevent overloading of the column. This in turn causes the detection limits to be higher in fast GC. This is a problem when working with trace levels of analytes.

It has been shown that using fast temperature programming is a better way than using faster flow rates to decrease the analysis times [84]. This parameter has been ignored in many studies, but it offers valuable time savings with some added benefits. Shorter columns with typical internal diameters (e.g. 0.25 mm) and film thicknesses can be used, without much loss in sample capacity.

It is important to ensure that the data collection rate is fast enough for peaks with low retention times in order to ensure good reproducibility of all peak parameters. For modern instrumentation, this is generally not a problem; for example, FID detectors are typically able to achieve a data acquisition rate of 50–250 Hz using the standard instrument configuration.

The data sampling rate of a typical quadrupole benchtop mass spectrometer is not always fast enough for very fast GC analyses, especially for quantitation. Typical spectral acquisition rates of scanning mass spectrometers, such as the ion trap, the quadrupole and the sector instruments, are limited to a maximum of ten to 20 spectra per second in the full-scan mode. This is just on the edge of applicability for fast GC. If faster detection is required, non-scanning TOF analysers are an alternative. TOFMS can provide up to 500 full spectra per second and allow accurate detection of peaks with peak widths in the millisecond range (very fast GC), while still providing high-quality spectra [85]. In terms of ultimate potential instrument performance, sensitivity is sacrificed for gains in speed in TOF [76]. In the literature, much of the discussion about fast GC-MS originates from the chromatographer's point of view, and a chromatographer tends to prefer baseline resolution between peaks. Although more selectivity in the separation can be beneficial in some respects, in other respects the time spent to resolve coeluting compounds by GC is wasted if the compounds can be adequately resolved by the MS detector. Mass-spectral deconvolution software is an effective and efficient tool to resolve coeluting peaks in GC-MS and thus is very important for fast GC-MS. Humans simply cannot conduct adequate back-
ground subtraction in a complex chromatogram, and a highly trained person could spend hours trying to do what an adequate deconvolution program can do in seconds. The future usefulness of fast GC-MS depends to some extent upon improvement of existing approaches and commercialisation of interesting

Fig. 15.8 Conventional GC (a) and fast GC (b) chromatograms of a lemon essential oil [77]. Copyright (2003) American Chemical Society
new techniques. Moreover, a greater emphasis is needed to rationalise overall laboratory operations and sample preparation procedures if fast GC-MS is to become implemented in routine applications [76].

15.4 Electronic Noses

In the food industry there exists an increased demand for fast, simple and sensitive methods of assessing volatiles, for identification, authentication, process control, and product blending or formulation. Since the quality of raw materials and processed products is determined frequently by the volatiles characteristic for a particular odour, objective methods for aroma and flavour evaluation are needed. By this means contaminations or off-flavours of such products could also be detected. Actually for this purpose human sensory panels of trained experts are used in the food and aroma industry, complemented by more objective, but time-consuming analytic methods, such as GC. In order to be able to interpret the laboratory analysis, those GC methods often have to be combined with MS or simultaneously used "sniffing" lines. However, even the most sophisticated analytic methods cannot fully replace the human nose, as only our olfactory sense can determine whether a compound is relevant to a specific odour. Therefore, it is not surprising that repeated efforts have been made over the years to introduce instruments operating on a similar principle to the human nose. The instruments comprise an array of electronic chemical sensors with partial specificity and an appropriate pattern-recognition system, capable of recognising simple or complex volatile mixtures. These systems would in most cases not replace but would complement conventional analyses of volatile compounds by sensory methods and by classic analytical techniques. The arrangements realised are often called "electronic noses" or "artificial noses" although the only aspect in common with our odour-sensing organ is the function. The operating principle, the number of sensors as well as the sensitivity and selectivity are, however, very different. This is why they should better be called "multisensor array technology".

Within the last few years, gas sensors together with associated pattern-recognition techniques have been used to differentiate and identify complex mixtures of volatile compounds. Potential areas of application include the food industry [86], perfumery, the chemical industry, the pharmaceutical industry, the tobacco industry [87], cosmetics [88], health and environmental control [89], where they are intended to be used for quality control of raw and manufactured products, monitoring of freshness and maturity, evaluating process effectiveness, prediction of shelf life, microbial pathogen detection [90, 91] and authenticity profiling. The major categories involved in the development of electronic noses and the principles of sensor design and technology will be described in the following section.
15.4.1 Catalytic or Metal Oxide Sensor

A catalytic or metal oxide sensor (MOS) consists of an electrically heated (250-450 °C) ceramic pellet upon which a thin film of tin(II) oxide doped with precious metals is deposited [92]. Tin(II) oxide is an n-type semiconductor and when oxygen adsorbs on the surface, one of the negatively charged oxygen species is generated, depending on the temperature. This results in the surface potential becoming increasingly negative and the electron donors within the material become positively charged. When an oxidisable material comes into contact with the sensor surfaces, the adsorbed oxygen is consumed in the resulting chemical reaction. This reduces the surface potential and increases the conductivity of the film. As a result the electrical resistance of the sensors will change. Disadvantageous are the relatively poor selectivity, which can be to some extent improved by dopants and temperature adjustment during the measurement, sensor drift over time, and finally the high power-consuming operation temperature.

15.4.2 Metal Oxide Semiconductor Field-Effect Transistor

The metal oxide semiconductor field-effect transistor (MOSFET) sensor device is based on a field-effect transistor with a catalytic metal as the gate contact. The gate voltage controls the current through the MOSFET device. The gas molecules will affect the voltage to the gate contact and thus change the current through the transistor. In a field-effect sensor, the interaction of gases with the catalytic gate metal induces dipoles or charges, which give an additional voltage to the gate contact. The choice of operation temperature, type of catalytic metal and structure of the metal influence the chemical reactions on the gate of the sensor, and thus the selectivity and sensitivity of the sensor.

15.4.3 Conducting Polymer Sensors

Polymer materials like polypyrrole and polyaniline are conducting (or semiconducting) and show a variation in conductivity with sorption of different gases and vapours. The sensor response is not necessarily a linear relationship between the analyte concentration and conductivity. Owing to their molecular structure, they show good sensitivities to polar compounds. The sensors display rapid adsorption and desorption at room temperature and specificity can be achieved by incorporating different metal ions in the structure of the polymer. Disadvantages include the reproducibility of fabrication (poor batch-to-batch reproducibility), strong humidity interference and the baseline drift over time.
owing to oxidation processes or changes in the conformation owing to exposure to inappropriate compounds.

15.4.4 Acoustic Wave Sensors

One of the first sensors to be introduced was the thickness-shear mode (TSM) sensor, which, if the substrate is quartz, may commonly be termed the quartz crystal microbalance (QMB) or bulk acoustic wave (BAW) sensor. The sensor consists of overlapping metal electrodes at the top and bottom. This type can be used with up to 10-MHz fundamental resonance frequency with a standing resonant wave being generated where the wavelengths are related to the thickness. As the thickness increases (e.g. owing to added mass by deposition on the surface), the wavelength increases and the frequency decreases. Mass-sensitive devices, such as QMB or SAW oscillators, can detect a change of mass accurately via resonance frequency shifts. In contrast to other sensor technologies, these kinds of transducers generate a fully digital electrical output signal with all its advantages to further signal processing (e.g. no analogue to digital conversion, fewer electromagnetic compatibility problems). In a SAW device, an acoustic wave propagates along the surface, whereas in a QMB crystal the acoustic wave propagates in the bulk. Compared with the SAW devices, the resonance frequency of QMB sensors is an order of magnitude lower, allowing a wider tolerance for temperature control of the oscillator electronics. When coated with gas-sensitive layers, both devices can detect gases or vapours. In contrast to SAW sensors, which use ultrathin layers to avoid damping of the oscillation, QMB sensors can also be coated with bulky layers. These coatings should have a high vapour permeability. SAW sensors can also be operated in the liquid phase and are theoretically more sensitive owing to their higher resonance frequency. In practice this advantage can often be compensated by using thicker coatings on the QMB devices. A large variety of chemical-sensitive materials can be deposited onto mass-sensitive devices. The long-term characteristics of these devices are mainly dependent on the ageing and/or bleeding of coating materials. Stationary phases used in GC columns (e.g. silicone, carbowax) have been optimised in respect to these features over the last 20 years. A QMB sensor, coated with these polymers, shows reproducible and stable behaviour for a wide range of chemical substances. The abilities and limitations of this type of sensor have been described in detail recently [93].

15.4.5 Mass Spectrometry Based Systems

While most of the commercially available gas sensors are based on one of the aforementioned four major sensor technologies, MS sensors are based on a measuring technique well known and widely used for almost 30 years: MS.
Volatile sample components are introduced into the mass spectrometer without separation, thus creating a mass spectrometric pattern of fragment ions that describes the mixture of volatiles in the headspace \([94-96]\). Each fragment ion represents a potential sensing element and the intensity of the fragment ion is equivalent to the sensor signal. Theoretically, when performing a full-scan measurement from, for example, \(m/z\) 50 to \(m/z\) 300, one can choose up to 251 sensors to form a sensor array. However, it is not useful to work with such a great number of sensors, as in most cases only a very small number of fragment ions is needed for setting up a sensor array. The choice of fragment ions that should be selected to build up an array is based on knowledge and has to be correlated with the sample properties to be determined.

Ion mobility spectrometry (IMS), which has the ability to separate ionic species at atmospheric pressure, is another technique that is useful for detecting and characterising organic vapours in air \([97]\). This involves the ionisation of molecules and their subsequent drift through an electric field. Analysis is based on analyte separations resulting from ionic mobilities rather than ionic masses. A major advantage of operation at atmospheric pressure is that it is possible to have smaller analytical units, lower power requirements, lighter weight and easier use.

Other MS-fingerprinting techniques that are in commercial development are based on atmospheric pressure ionisation (API), resonance-enhanced multiphoton ionisation (REMPI) TOF and proton-transfer reaction (PTR). They are rapid, sensitive and specific and allow measurements in real time and may play an increasingly important role in the future development of electronic noses and tongues.

### 15.4.6 Other Sensor Technologies

Apart from the aforementioned most frequently used sensor technologies, also selective electrochemical sensor combinations have been commercialised for use in dedicated applications. The combination of electrochemical CO, H\(_2\)S, SO\(_2\) and NH\(_3\) sensors was used for quality and freshness control of foods like fish \([98]\) and meat \([99]\). Combinations of MOSs and MOSFETs supplemented with a selective IR absorption sensor for carbon dioxide and a humidity sensor for measuring relative humidity were also described \([100]\).

One of the technologies which may become relevant for gas sensing is bundled fibre optics, through which fluorescence is measured from photodeposited polymer-sensing elements. On one end of a fibre optic bundle, as many as 30 small regions of polymer fluorescent dye mixtures are photodeposited. A flash of light at an excitation wavelength is applied to the other end of the fibre optic, and fluorescence intensity at selected wavelengths from the polymer/dye mix is subsequently measured back through the fibre optic. Different polymer/dye combinations interact with gases differently, such that upon exposure to a given sample, the different regions or sensors provide unique information \([101]\).
Advances in the production, immobilisation and characterisation of mammalian olfactory receptors led to the development of biosensors where isolated olfactory binding proteins were deposited on the surface of QMBs [102, 103] or were connected to nanoelectrodes [104]. Although still at the development stage, such an array-type device coated with different olfactory receptors will be a powerful and useful tool for detecting and discriminating odorants in the future.

15.4.7 Data Processing

A generalised structure of an electronic nose is shown in Fig. 15.9. The sensor array may be QMB, conducting polymer, MOS or MS-based sensors. The data generated by each sensor are processed by a pattern-recognition algorithm and the results are then analysed. The ability to characterise complex mixtures without the need to identify and quantify individual components is one of the main advantages of such an approach. The pattern-recognition methods may be divided into non-supervised (e.g. principal component analysis, PCA) and supervised (artificial neural network, ANN) methods; also a combination of both can be used.

PCA reduces multidimensional, partly correlated data, to two or three dimensions. Projections are chosen so that the maximum amount of information is retained in the smallest number of dimensions. This technique allows the similarities and differences between objects and samples to be better assessed [105].

A neural network is a program that processes data like (a part of) the nervous system. Neural networks are especially useful for classification problems and for function approximation problems which are tolerant of some imprecision, which have lots of training data available, but to which hard and fast rules (such as laws of nature) cannot easily be applied.
Neural networks are trained with complete data sets consisting of input and output data. Typically one starts with a random configuration and calculates output data from the given input data. One compares the calculated output data with the output data of the complete data set and tries to minimise the error of the output data by varying the parameters of the network. When the network has learned the complete data sets, one takes an independent collection of complete data sets to test the generalisation capability of the network. Both collections of complete data sets must be large enough and correctly distributed within the range of possible data. Perhaps the greatest advantage of ANNs is their ability to be used as an arbitrary function approximation mechanism which learns from observed data. However, using them is not so straightforward and a relatively good understanding of the underlying theory is essential. The danger is that the network overfits the training data and fails to capture the true statistical process generating the data, resulting in worse predicting ability.

15.4.8 Applications, Potential and Limitations

Most publications deal with the application to foods (Table 15.3), but published studies are also available covering other products, such as tobacco, cosmetics, health diagnostics and the environment [86].

The feasibility and limitations of using multisensor array systems in food and aroma applications will be discussed with an application intended to discriminate hop varieties [149] by way of example.

The sensor responses generated in a measurement result from physical and/or chemical interactions between the sensors and the volatile compounds present in the headspace above the measured sample. By using a QMB sensor system with an array of six sensors, good discrimination between three hop varieties can be observed (Fig. 15.10a). In this example only 12 measurements per sample were analysed. The distance between clusters is reduced if the data set is increased to 50 measurements per sample (Fig. 15.10b).

The reason for this effect has to be attributed to a better and adequate ratio between sample size and array dimensionality. For a significant clustering of the patterns, with an array of six sensors a sample size of at least 18 is required [149, 184]. As a consequence, the discrimination based on only 12 measurements has poor statistical relevance. Most of the applications with sensor arrays found in the literature do not consider this fact; frequently discriminations with 12–32 sensors in an array and with a sample size of three to four are described. All of them are of limited feasibility with concurrent poor validation, especially in terms of reproducibility and predictive ability. In other words, if there are not enough calibration measurements one can separate data in a predetermined way, but will fail to verify the result using independent test samples.

A great disadvantage of MOS, MOSFET, conducting polymer and QMB sensor arrays is that system-to-system matching is not possible in practice, as can
be seen in Fig. 15.11. A discrimination of hop varieties with QMB and MOS arrays is partly obtained, but the results are not comparable. The hop variety Tradition, for example, is well separated from other varieties with the MOS sensor, whereas with the QMB sensor overlapping with other varieties is observed. The different responses and sensitivities of the sensors for chemical compounds is the reason for non-comparable discriminations when different sensor systems are used; therefore, a standardisation of these electronic noses is not possible.

Fig. 15.10 Discrimination of hop varieties with six quartz crystal microbalance (QMB) sensors with 12 (a) and 50 (b) measurements per sample. N Nugget, S Select, M Magnum, P Perle, T Tradition, B Northern Brewer

Fig. 15.11 Discrimination of six hop varieties (see Fig. 15.10) by means of metal oxide sensor (MOS) and QMB sensor arrays
The interesting question now is whether the sensors classified the different hop samples on the basis of interaction with substances in the headspace which are highly correlated with the varieties. The GC analyses clearly show that myrcene is the main component in the headspace and is therefore responsible for the signals generated by the sensors. But the content of myrcene in hops is highly dependent on climatic, soil, growth and processing conditions, and cannot be regarded as a specific indicator for a variety assessment. Therefore, not the different hop varieties, but the different content of myrcene in the samples was discriminated. Frequently, authors tend to correlate an observed discrimination with the property they wanted to measure, without any additional chemical information. Electronic noses as commonly employed do not allow for chemical differentiation. Owing to the unspecific nature of the sensors, the reasons for a successful discrimination of samples are usually unknown.

In order to correlate a discrimination to the different varieties, a sensor system that selectively interacts with variety-specific compounds in the headspace is needed. The GC analysis of the essential oil reveals that there are some minor volatile compounds, which can be used for a differentiation of different hop varieties (e.g. Nugget and Tettnanger), as shown in Fig. 15.12.

By choosing appropriate fragment ions a virtual sensor array based on a mass spectrometer in single ion monitoring mode can be implemented to discriminate the hop varieties (Fig. 15.13) without appreciable interference of the main component myrcene. In contrast to the analyses performed with QMB and MOS sensors, the discrimination obtained with the MS sensor is based on chemical knowledge and not on assumptions.

![Fig. 15.12 Expanded region of a GC chromatogram of the hop varieties Nugget and Tettnanger](#)
15.4.9 Conclusions

The electronic nose technology applied to food must be regarded as being in its early stage. There is rapidly advancing research and development going on both for sensors and instrument hardware and software in order to enhance selectivity, sensitivity and reproducibility of the gas sensors. Much effort is also being put into solving the drift problem of the sensors by increasing their stability and lifetime, and into developing improved mathematical algorithms for drift counteraction, automatic calibration and standardisation, as well as transferability between gas sensor array instruments.

The performance of common multisensor arrays is ultimately determined by the properties of their constituent parts. Key parameters such as number, type and specificity of the sensors determine whether a specific instrument is suitable for a given application. The selection of an appropriate set of chemical sensors is of utmost importance if electronic nose classifications are to be utilised to solve an analytical problem. As this requires time and effort, the applicability of solid-state sensor technology is often limited. The time saved compared with classic analytical methods is questionable, since analysis times of electronic nose systems are generally influenced more by the sampling method utilised than the sensor response time [185].

Common electronic noses are so called as they are often aimed at detection of odorous compounds; it is generally not clear that discriminations are based on odorous rather than non-odorous, and possibly incidental, components of the headspace. In the headspace of a food sample, odorants contributing to the flavour may be present in low concentrations, whereas non-odorous molecules can be present in much larger numbers and higher concentration. In such cases,
### Table 15.3 Selected applications of electronic nose systems to different food products

<table>
<thead>
<tr>
<th>Product</th>
<th>Type of application</th>
<th>Sensor technology</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grains</td>
<td>Off-odour caused by microbial infection</td>
<td>MOS</td>
<td>[107]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>MOSFET, MOS, IR</td>
<td>[108, 109]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CP</td>
<td>[110]</td>
</tr>
<tr>
<td>Meat</td>
<td>Quality assessment, lipid oxidation, fermentation, storage</td>
<td>MOSFET, MOS</td>
<td>[111, 112]</td>
</tr>
<tr>
<td></td>
<td>spoilage/shelf life</td>
<td>MOSFET, MOS, IR</td>
<td>[110, 103]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CP</td>
<td>[114, 115]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CO, H₂S, H₂O, NH₃</td>
<td>[99]</td>
</tr>
<tr>
<td>Fish</td>
<td>Quality assessment, lipid oxidation, spoilage, freshness,</td>
<td>MOSFET, MOS</td>
<td>[116]</td>
</tr>
<tr>
<td></td>
<td>storage</td>
<td>MOS</td>
<td>[117]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CP</td>
<td>[118–120]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CO, H₂S, H₂O, NH₃</td>
<td>[121, 122]</td>
</tr>
<tr>
<td>Dairy products</td>
<td>Flavour quality, cheese characterisation, heat treatment,</td>
<td>BAW, MOS</td>
<td>[125]</td>
</tr>
<tr>
<td></td>
<td>flavour differences, off flavours, microbial contaminants</td>
<td>CP</td>
<td>[126, 131–133, 139]</td>
</tr>
<tr>
<td>Fruits</td>
<td>Flavour quality, harvest dates, storage, maturity, processing</td>
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<tr>
<td></td>
<td></td>
<td>MOS</td>
<td>[138]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CP</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>QMB, CP, MS</td>
<td>[139]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>MOS, CP</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>CP</td>
<td>[128, 130, 136, 140–144]</td>
</tr>
<tr>
<td>Alcoholic</td>
<td>Wines, spirits, origin, variety, barrel ageing, cork taint</td>
<td>MOS, CP, QMB</td>
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<tr>
<td>beverages</td>
<td></td>
<td>MS</td>
<td>[151]</td>
</tr>
<tr>
<td>Beer</td>
<td>Characterisation</td>
<td>MS</td>
<td>[152–155]</td>
</tr>
<tr>
<td>and hops</td>
<td>of aroma, ageing, raw materials, hop varieties</td>
<td>MOS</td>
<td>[156]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>MS</td>
<td>[157]</td>
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<tr>
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<td></td>
<td>QMB, MOS, MS</td>
<td>[158]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>MOS</td>
<td>[159, 160]</td>
</tr>
<tr>
<td>Spices</td>
<td>Characterisation, differentiation, composition of mixtures</td>
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<td>[149]</td>
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<tr>
<td></td>
<td>microencapsulation, γ-irradiation</td>
<td>CP</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>MOS</td>
<td>[161]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CP</td>
<td>[162]</td>
</tr>
<tr>
<td></td>
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<td>QMB, MOS, QMB</td>
<td>[163, 167, 168]</td>
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<td>QMB</td>
<td>[93, 164, 165]</td>
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<td>Oxidation, rancidity, vinegary defects, distinguish different</td>
<td>MOSFET, MOS</td>
<td>[94, 166]</td>
</tr>
<tr>
<td></td>
<td>qualities, shelf life, geographical origin?</td>
<td>SAW</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>MOS</td>
<td>[169]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CP</td>
<td>[170]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CP, MOS, MOSFET</td>
<td>[171, 172]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>QMB</td>
<td>[172]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>MS</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td>QMB</td>
<td></td>
</tr>
<tr>
<td>Coffee</td>
<td>Discrimination, roasting</td>
<td>CP</td>
<td>[163]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>MOS</td>
<td>[164]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>REMPI-TOFMS</td>
<td>[175]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>MOS, CP, QMB</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>QMB</td>
<td>[176]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>MOS</td>
<td>[177, 178, 180]</td>
</tr>
<tr>
<td>Packaging</td>
<td>Retained solvents, printing inks, colouring agents, foil</td>
<td>MOSFET, MOS, IR</td>
<td>[179]</td>
</tr>
<tr>
<td></td>
<td>adhesives</td>
<td>QMB</td>
<td>[181]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>MOSF, CP, QMB</td>
<td>[182]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>QMB, MOS, MS</td>
<td>[183]</td>
</tr>
</tbody>
</table>

MOS metal oxide sensor, MOSFET metal oxide semiconductor field-effect transistor, IR infrared, CP conducting polymer, QMB quartz crystal microbalance, IMS ion mobility spectrometry, BAW bulk acoustic wave, MS mass spectrometry, SAW surface acoustic wave, REMPI-TOFMS resonance-enhanced multiphoton ionisation time-of-flight mass spectrometry
MS-based systems have considerable advantages over the commonly used gas-sensor arrays, particularly in terms of selectivity, adaptability, sensitivity and standardisation. Array selection and deselection can be done rapidly by changing the scanning method and/or simply by changing the fragment ions used for pattern recognition. Furthermore, taking into account that fragment ions contain chemical information about the sample, the information that can be obtained with an electronic nose improves substantially with the MS-based devices. An optimal instrument configuration also allows the same instrument to be used as a rapid screening tool (electronic nose) and also as a research tool for revealing further chemical information about doubtful samples. A great advantage is obtained with soft ionisation methods like PTR-MS or REMPI because molecular information is easier to deconvolute in the case of overlapping fragments or parent ions.

Although common electronic noses are generally not suitable for odour assessment, they can be successfully used in applications where the main components in the headspace are directly correlated with the property to be determined (e.g. quality of spice mixtures) or in cases where substances are formed and released into the headspace, for example owing to oxidation processes, fermentation, microbial contamination, thermal treatment, etc.

15.5 Time-Resolved Analysis of Volatile Organic Compounds

Over the last decade, interest in release and delivery of VOCs has been steadily growing, with a particular focus on food, environmental and medical applications [186–190]. Consequently, considerable effort was invested to develop analytical methods capable of capturing such dynamic VOC release processes (Fig. 15.14) [179, 191]. This led to improvements in electronic sensor methods (often termed “electronic noses”) [192].

One other approach is direct-inlet MS. A prerequisite for mass analysis is ionisation, a process that critically influences the sensitivity and selectivity of the experiment. Electron impact ionisation (EI) causes considerable fragmentation. Because of overlapping fragment and parent ions, the molecular information is difficult to deconvolute, and little chemical information can be extracted.

Therefore, application of direct-inlet MS for monitoring complex mixtures of VOCs requires using ionisation techniques which produce little or no fragmentation (soft ionisation). Chemical ionisation in combination with a quadrupole mass filter, either in atmospheric pressure chemical ionisation MS (APCI-MS) [188, 189] or in PTR-MS [193–195], have been successfully applied to VOC analyses. The advantages and limitations of direct-inlet MS with soft-ionisation approaches have been discussed [196].

One particularly well-performing technique is PTR-MS [193–195]. On-line trace-gas analysis by proton transfer [197] has become a powerful approach, mainly owing to the higher sensitivity and lower ionisation-induced fragmen-
15.5 Time-Resolved Analysis of Volatile Organic Compounds

Time-resolved headspace analysis

Gas Sensors

Direct Inlet MS

Hard Ionisation
(strong fragmentation)

Soft Ionisation
(little fragmentation)

Electronic Nose

Electron Impact

Chemical Ionisation

Laser Ionisation

 PTR-MS

REMPI-TOFMS

---

Fig. 15.14 Analytical techniques for time-resolved headspace analysis. An electronic nose can be used as a low-cost process-monitoring device, where chemical information is not mandatory. Electron impact ionisation mass spectrometry (EI-MS) adds sensitivity, speed and some chemical information. Yet, owing to the hard ionisation mode, most chemical information is lost. Proton-transfer-reaction MS (PTR-MS) is a sensitive one-dimensional method, which provides characteristic headspace profiles (detailed fingerprints) and chemical information. Finally, resonance-enhanced multiphoton ionisation (REMPI) TOFMS combines selective ionisation and mass separation and hence represents a two-dimensional method. (Adapted from [190])

15.5.1 Proton-Transfer-Reaction Mass Spectrometry

PTR-MS combines a soft, sensitive and efficient mode of chemical ionisation, adapted to the analysis of trace VOCs. Briefly, headspace gas is continuously introduced into the chemical ionisation cell, which contains besides buffer-gas a controlled ion density of H$_3$O$^+$. VOCs that have proton affinities larger than water (proton affinity of H$_2$O is 166.5 kcal/mol) are ionised by proton transfer from H$_3$O$^+$, and the protonated VOCs are mass-analysed. The chemical ionisation source was specifically designed to achieve versatility, high sensitivity and little fragmentation, and to allow for absolute quantification of VOCs. To
achieve these targeted specifications, the generation of the primary H$_3$O$^+$ ions and the chemical ionisation process—proton transfer from H$_3$O$^+$ to VOCs—are spatially and temporally separated and individually controlled. This allows (1) maximising signal intensity by increasing the generation of primary reactant ions, H$_3$O$^+$, in the ion source, (2) reducing fragmentation and clustering by optimising the conditions for proton transfer in the drift tube and (3) quantifying VOCs from measured count rates.

The four key features of PTR-MS can be summarised as follows. First, it is fast. Time dependent variations of headspace profiles can be monitored with a time resolution of better than 1 s. Second, the volatile compounds do not experience any work-up or thermal stress, and very little fragmentation is induced by the ionisation step; hence, measured mass spectral profiles closely reflect genuine headspace distributions. Third, measured mass spectral intensities can be directly related to absolute headspace concentrations, without calibration or use of standards. Finally, it is not invasive and the process under investigation is not affected by the measurements. All these features make PTR-MS a particularly suitable method to investigate fast dynamic process.

15.5.1.1 Technical Features

PTR-MS was introduced in 1993 by Lindinger and co-workers at the university of Innsbruck. A schematic drawing of the apparatus is given in Fig. 15.15. Here, only a brief description will be given. A more detailed discussion of the technical aspects of PTR-MS has been published in a series of review papers [193–195].

Primary (reactant) ions A$^+$, generated in a hollow cathode ion source, travel through a buffer gas within the drift tube, to which the reactant gas (VOC) is added in small amounts, so that the density of the buffer gas is much larger than the density of the VOC. On their way through the reaction region, ions perform many non-reactive collisions with buffer gas atoms or molecules; however, once they collide with a reactant gas particle, they may undergo a reaction:

$$A^+ + \text{VOC} \rightarrow \text{products.} \quad (1)$$

When H$_3$O$^+$ is used as the proton donor, most of the organic trace components R in air are ionised by proton-transfer processes:

$$\text{H}_3\text{O}^+ + \text{VOC} \rightarrow k \rightarrow \text{VOC.H}^+ + \text{H}_2\text{O.} \quad (2)$$

These reactions are invariably fast, whenever they are exoergic, with rate coefficients, k, close to the collisional limiting values, $k_0 \approx 10^{-9}$ cm$^3$/s$^1$ [197]. Water has a proton affinity of 7.22 eV (166.5 kcal/mol), and common organic mole-
Molecules have proton affinities in the range from 7 to 9 eV (161–208 kcal/mol), as shown in Table 15.4. Hence, most of the relevant proton-transfer reactions involving $\text{H}_2\text{O}^+$ are slightly exoergic, and $\text{H}_2\text{O}^+$ will perform proton-transfer reactions with nearly any kind of VOC in the headspace of food products. However, $\text{H}_2\text{O}^+$ does not react with the natural components of air such as $\text{O}_2$, $\text{N}_2$, $\text{CO}_2$, $\text{CO}$ or others (see Table 15.4). The exoergicity of the proton-transfer reaction with most VOCs, however, is low enough that breakup seldom occurs. On the basis of this ionisation principle, a PTR-MS setup was developed applicable to trace-gas analysis, and aimed at speed, sensitivity, versatility and simple handling.

The example shown in Fig. 15.16 was obtained by reconstituting a powdered beverage with hot water while measuring the headspace VOCs on-line by PTR-MS. It shows the relative ratio of the compounds released into the headspace and their dynamic behaviour. However, it is hardly possible to assign the mass traces to individual VOCs. For that, coupling of PTR-MS with GC-MS is required, which will be discussed in the next section.

---

**Fig. 15.15** The PTR-MS apparatus. It consists of a series of three main chambers. In the first chamber, $\text{H}_2\text{O}$ is introduced and protonated in an electrical discharge to form $\text{H}_3\text{O}^+$. These ions are then driven by a small field through an orifice into the drift tube (chemical ionisation chamber). Coaxial to this orifice, neutral volatile organic compounds (VOCs) are introduced into the drift tube and collide at thermal energies with $\text{H}_3\text{O}^+$. VOCs with proton affinities exceeding 166.5 kcal/mol are ionised by proton transfer from $\text{H}_3\text{O}^+$ and are accelerated out of the drift tube into the quadrupole mass filter and onto the detector. (Adapted from [190])
Table 15.4 Proton affinities of the constituents of clean air and of various volatile organic compounds. All volatile organic compounds with a higher proton affinity than H₂O (166.5 kcal/mol) will be protonated with a very high efficiency when colliding with H₃O⁺. This is the case for most of the volatile organic compounds in the headspace of coffee, with the exception of the natural constituents of clean air. In contrast, if NH₃ is used as a chemical ionisation agent, only compounds with a proton affinity exceeding 204.0 kcal/mol are ionised (below dotted line). (Adapted from [190])

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Proton affinities (kcal/mol)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Name</td>
<td>Formula</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>Water</td>
<td>H₂O</td>
</tr>
<tr>
<td>Butane</td>
<td>C₄H₁₀</td>
</tr>
<tr>
<td>Hydrogen sulphide</td>
<td>H₂S</td>
</tr>
<tr>
<td>Hydrogen cyanide</td>
<td>HCN</td>
</tr>
<tr>
<td>Formic acid</td>
<td>HCOOH</td>
</tr>
<tr>
<td>Propane</td>
<td>C₃H₆</td>
</tr>
<tr>
<td>Benzene</td>
<td>C₆H₆</td>
</tr>
<tr>
<td>Methanol</td>
<td>C₂H₅OH</td>
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<tr>
<td>Acetaldehyde</td>
<td>CH₃COH</td>
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<tr>
<td>Acetonitrile</td>
<td>CH₂CN</td>
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<tr>
<td>Ethanol</td>
<td>C₂H₅OH</td>
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<tr>
<td>Furane</td>
<td>C₄H₄O</td>
</tr>
<tr>
<td>2,3-Butanedione</td>
<td>C₄H₈O₂</td>
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<tr>
<td>Acetone</td>
<td>CH₃COCH₃</td>
</tr>
<tr>
<td>2,3-Methylbutanal</td>
<td>C₆H₁₄O</td>
</tr>
<tr>
<td>Ammonia</td>
<td>NH₃</td>
</tr>
<tr>
<td>Pyrrole</td>
<td>C₃H₃N</td>
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Table 15.4 (continued) Proton affinities of the constituents of clean air and of various volatile organic compounds.

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Formula</th>
<th>Proton affinities (kcal/mol)</th>
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<tr>
<td>Oxazole</td>
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<td>Pyrazole</td>
<td>C₅H₅N₂</td>
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<tr>
<td>Pyridine</td>
<td>C₅H₅N</td>
<td>220.8</td>
</tr>
<tr>
<td>Trimethylamine</td>
<td>C₅H₇N</td>
<td>225.1</td>
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</tbody>
</table>

Fig. 15.16 Ion traces (in m/z) of VOCs released upon reconstitution of an instant beverage and analysed on-line by PTR-MS

15.5.1.2 Coupling of Proton-Transfer-Reaction Mass Spectrometry with Gas Chromatography–Mass Spectrometry

The success of PTR-MS triggered interest in further improving its performance. Indeed, PTR-MS is a one-dimensional technique, and ions from a complex headspace, e.g. coffee, can often only be tentatively assigned. Ions from different compounds (parent and fragment ions) can overlap in PTR-MS and prevent an unambiguous identification of VOCs in a complex mixture [198]. There-
fore, the attempt has been made to address this problem and propose an extension of PTR-MS which allows for an unambiguous identification of headspace compounds. This is achieved by coupling GC with simultaneous PTR-MS and EI-MS detection. In this chapter, we can only introduce the basic features of the new setup: a technical and analytical extension of PTR-MS which removes this shortcoming, while preserving its salient and unique features. Combining separation of VOCs by GC with simultaneous and parallel detection of the GC effluent by PTR-MS and EI-MS, an unambiguous interpretation of complex PTR-MS spectra becomes feasible. A more detailed description of characteristic performance parameters, such as resolution, linear range and detection limit, has been published in a recent paper [199].

As an example, the novel setup was applied to the characterisation of coffee headspace as a complex food system. Basically, an aliquot of the headspace is trapped in defined time periods on several Tenax® adsorbents for characterisation by GC-MS. Figure 15.17 shows the simultaneously recorded total ion counts of the EI-MS (top frame) and PTR-MS (bottom frame) for VOCs trapped on the first Tenax® cartridge. The GC-separated pure compounds are identified based on MS spectra obtained at 70 eV and the retention index of the reference compounds. (Adapted from [199])
by comparison of their EI-MS fragmentation patterns with the Wiley database (Wiley 7th edition) as well as their retention indices obtained with reference compounds. The PTR-MS spectrum allows the PTR-MS fragmentation pattern of the GC-separated pure compounds to be identified.

GC traces over the entire 40 min of the GC run are shown in Figs. 15.18 and 15.19, for the compounds desorbed from Tenax® cartridge no. 1 (trapping time window between 1 and 3 min). The data reveal that the PTR-MS ion signal at m/z 111 is a superposition of ions originating from two different compounds, i.e. 2-acetyl furan and 5-methyl furfural, contributing with 29 and 71%, respectively, to the total ion peak intensity at m/z 111. Similarly, the PTR-MS ion signal at m/z 87 is a superposition of 57% 2-methyl-1-propanal and 39% 2-butanone, with traces from 4-methyl-2-pentanone and 2-methyl tetrahydrofuran-3-one (2% each). While the single PTR-MS traces shown in Fig. 15.18 represent a superposition of several compounds, a series of PTR-MS ion traces are shown in Fig. 15.19 that are nearly pure (more than 89%), indicating that essentially only one single compound contributes to the ion signal (with only traces from other VOCs). Hence, in an on-line PTR-MS measurement of coffee headspace, the ion masses at m/z 68, 75, 80 and 95 can be assigned to pyrrole, acetol, pyridine and

![Fig. 15.18 Unambiguous identification of the molecules assigned to the trace ions. This identification is only valid for the first 120-s period of Tenax® trapping. (Adapted from [199])](image-url)
2-methylpyrazine, respectively. The coupling of PTR-MS with GC-MS, as introduced here, allows identification and quantification of the VOCs that contribute to a single PTR-MS ion signal.

15.5.2 Resonance-Enhanced Multiphoton Ionisation Time-of-Flight Mass Spectrometry

Selective and time-resolved monitoring can be achieved by REMPI at 266 nm coupled to a direct-inlet TOFMS device. Selectivity was introduced into the ionisation step by resonant ionisation at a fixed UV laser wavelength. The photoexcitation energy scheme for REMPI is illustrated in Fig. 15.20.

Depending on molecular resonances, VOCs with an optical (electronic) absorption at 266 nm absorb a laser photon, while those transparent at 266 nm remain in the ground state. The width of optical absorptions is given by the ground-state population, and broadens with the molecule's temperature, which itself depends on the expansion conditions at the inlet system.
Since an effusive molecular beam was used (no cooling), a range of rotational and vibrational states was populated, resulting in broad absorption bands. Consequently, a range of compounds may be ionised simultaneously, owing to overlapping absorption bands [200]. Technical reviews on REMPI can be found in the literature [200–202].

In a typical REMPI scheme, molecules absorb a first photon and are excited into a UV electronic state. These excited molecules are subsequently ionised by absorbing a second photon. For effective and selective REMPI detection, the following conditions have to be fulfilled:

1. **Resonance condition:** the molecule has a UV-active excited state, whose energy corresponds to the energy of the laser photon.
2. **Lifetime condition:** the excited state has a lifetime which is long enough for it to absorb a second photon for ionisation.
3. **Ionisation condition:** the energy of two photons is equal to or higher than the ionisation energy of the molecule.

The on-line VOC sampling depicted in Fig. 15.21 gives a schematic overview of the experimental setup, to illustrate the sampling of the roaster gas and the introduction of the volatiles into the TOF mass spectrometer [203]. A quartz tube with a passivated inner surface of 10-mm inner diameter was used to sample gas from the roaster. The tube reached about 2 cm into the rotating drum. A constant off-gas sampling stream of 1.5 l/min was pumped through the sampling system. A quartz wool paper filter was integrated into the tube to prevent solid contamination such as dust or silver skins reaching the capillary inlet.
system. All sampling lines were heated to 250 °C, to minimise condensation of low-volatile compounds.

A typical REMPI at 266 nm mass spectrum is shown Fig. 15.22, obtained by roasting 80 g of *Arabica* coffee at 225 °C. The laser power density was adjusted to $10^6-10^7$ W/cm² in order to avoid non-resonant ionisation processes. The spectrum contains predominantly molecular ions. Chemical assignment of the ion peaks was based on three distinct pieces of information: the literature on coffee flavour compounds [204], the mass as observed in TOFMS and optical absorption properties. With this information, many volatiles observed in Fig. 15.22 were unambiguously identified.

A full three-dimensional representation—mass, time, intensity—of a typical roasting process at 200 °C, recorded at 10 Hz by REMPI at 248 nm is shown in Fig. 15.23, panel a [179]. Characteristic cross-sections through the three-dimensional surface are given in Fig. 15.23, panels b and c. Figure 15.23, panel b gives a cross-section of the roast gas composition at a fixed time (approximately 12 min). In Fig. 15.23, panel c two cross-sections at fixed masses $m/z$ 94 and $m/z$ 150 are shown, corresponding to $t-I$ profiles of phenol and 4-vinylguaiacol.

![Roaster and Time-of-Flight Mass Spectrometer](image)

**Fig. 15.21** The experimental setup including the laboratory-scale coffee roaster with a sampling unit and a laser mass spectrometer. The homebuilt mobile device consisted of a Reflectron TOFMS analyser, an effusive beam inlet system and a built-in laser operated at 266 nm (Continuum Nd: YAG laser SURELIGHT™, 266 nm). (Adapted from [204]).
15.5 Time-Resolved Analysis of Volatile Organic Compounds

Fig. 15.22 On-line REMPI-TOFMS (at 266 nm) analysis of roast gas while roasting 80 g *Arabica* coffee. 

**a** The full-time–mass–intensity three-dimensional plot as recorded during roasting. 

**b** A time–intensity cross-section from **a** at a fixed time (medium roast level). The three phenolic VOCs, phenol (m/z 94), guaiacol (m/z 124) and 4-vinylguaiacol (150 m/z), are efficiently ionised at 266 nm. In addition, furfurylacohol (m/z 96), dihydroxybenzene (m/z 110), indol (m/z 117) and caffeine (m/z 194) were also detected. (Adapted from [203])
Fig. 15.23  a Three-dimensional REMPI at 248 nm TOFMS mass spectrum of coffee roasting off-gas while roasting in a steel cylinder at 200 °C. The three dimensions are mass, time and intensity. b Cross section of a at a fixed time. c Time-intensity REMPI at 248 nm TOFMS profiles of phenol (m/z 94) and 4-vinylguaiacol (m/z 150), corresponding to two cross-sections from a at fixed masses. (Adapted from [179])
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