Predicting organoleptic scores of sub-ppm flavour notes. Part 1. Theoretical and experimental details

The Analyst

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Most existing electronic nose systems have limited commercial application since they only provide a relative description of the flavour under investigation, rather than one against a universal standard. However, the development of a set of universally accepted standards for flavour description has been problematic due to the lack of any comprehensive model relating the molecular structure of an odorant with its flavour-impact during the act of perception. Instead, industries have tended to develop their own flavour models (flavour terminology systems) for specific consumer products that are based upon practical experience of a particular foodstuff, cosmetic, or beverage. We report here on the novel application of chemical multi-sensor arrays to the prediction of organoleptic flavour notes, as defined under a specific terminology system suitable for describing and communicating specific flavours. A novel odour mapping scheme is proposed that may be applied generally to multi-sensor arrays and provides more detailed characterisation of odour quality than is currently achievable. As part of our study, a flow injection analyser (FIA) system has been developed that combines chemical and electronic hardware driven by a microcomputer to achieve accurate and independent control over odour-stream temperature, flow-rate and flow profile, sensor head temperature, and sample times. An array of 24 conducting polymer sensors (11 different types) is used within the FIA system giving an overall experimental coefficient of variation below 7%. The application of this odour mapping technique is demonstrated by way of an experimental study, using the FIA system reported here. The details for this study are given in Part 1, and the computational analysis of the data is carried out in Part 2 (T. C. Pearce and J. W. Gardner, *Analyst*, 1998, **123,** 2057.

Flavour complexity

Flavour is complex in terms of its many chemical and sensory components. Prior to the advent of instrumental techniques in flavour analysis the flavour of foodstuffs was only attributed to a small group of key impact chemicals. The use of gas chromatography (GC) and mass spectrometry (MS) analysis demonstrates that, in fact, most naturally occurring flavours comprise hundreds of different chemical compounds existing in a complex mixture.

Aishima and Nakai have described flavour in terms of its sensory and chemical components in the following equations[1]

$$\text{flavour} = a_1\,(\text{aroma}) + a_2\,(\text{taste}) + a_3\,(\text{texture}) + a_4\,(\text{colour}) + a_5\,(\text{sound}) + a_6\,(\text{temperature}) \quad (1)$$

$$\text{flavour} = b_1X_1 + b_2X_2 + \cdots + b_iX_i + \cdots + b_mX_m \quad (2)$$

where the sensory components are given in terms of the coefficients, a_i, and summed in eqn. (1) to form the overall subject's perception of flavour. This linear model has severe limitations in providing an adequate picture of the quality of flavour. In particular, the values of a_i (*i.e.*, the relative impact of each of the sensory modalities) are found to vary widely for different flavours and also for different individuals. Furthermore, no account is taken in the model of the many sensory confusions that occur between each of the sensory modalities in normal adults, termed synæsthesia.

Flavour can also be explained in terms of its chemical components defined in eqn. (2). Here the concentrations, X_i, of chemical species, i, have a sensory impact determined by the coefficients, b_i, and are summed over m components (typically $m > 100$ for complex odours) to provide the flavour mixture. Again, this linear model does not account for the additive, synergistic, antagonistic, and compensative effects that can occur between flavour volatiles existing in such a mixture. The high dimensionality and inter-dependency of flavour components (both sensory or chemically based) are two factors that have prevented the development of a unified theory of flavour analysis.

A unified theory of flavour (or odour) analysis would be able to explain and predict the sensory impact of simple flavours (odours), both individually and in combination, in terms of their chemical constituents. Clearly, such a theory would be of enormous benefit to, amongst others, the food and beverage industries, permitting the development of unambiguous standards by which to describe all complex odours and flavours. Unfortunately, current theories are only able to explain a small subset of the total possible structure–activity relationships (SAR) existing between the chemical and sensory domains.[2–4]

Research in this field has not only been hampered by the high-dimensionality and inter-dependency of the variables involved, but also by a number of other contributing factors; there are real practical difficulties involved in the purification of the flavour stimuli. It is impossible to carry out control of the stimulus in a linear or even continuous fashion, and perhaps more importantly, no single underlying molecular determinant has yet been isolated for flavour that can account for all, or even a significant portion of SAR effects during flavour perception (see Rossiter[5] for a current review of SAR studies). Notable past studies have led to the conclusion that over 20 different molecular determinants are likely to be involved in the transduction of odour signals alone.[6,7] These factors combined with the lack of unambiguous vocabulary for describing odours

and flavours, as well as incomplete knowledge of the receptor mechanisms and subsequent neural processing implicated in olfactory information processing, present significant challenges to be overcome if a unified theory of odour analysis is to be developed in the future.

Flavour analysis

In view of the lack of any unified theory of flavour (or odour) two very disparate approaches are currently taken to flavour analysis—sensory and instrumental analysis. On the one hand, instrumental techniques [such as GC, LC (Liquid Chromatography), MS, and NMR (Nuclear Magnetic Resonance)] tend to be reserved for flavour research, principally due to their high capital and operating costs. In particular, instrumental techniques are incapable of directly measuring the quality of flavour, since no account is made of the psychophysical effects or flavour-impact these compounds will have during perception. Furthermore, since vastly different sensitivities in humans are displayed to various flavour active chemicals within a complex mixture, the estimated concentrations of flavour active compounds, as calculated from chromatogram peaks, mean little without some qualitative context.

Sensory analysis, on the other hand, still provides the key technique in flavour analysis today, due to the superb sensitivity and discrimination of the chemical senses. Sensory-based flavour analysis is carried out by teams of highly skilled tasters who work to rigorous testing protocols, such as the triangular taste test, the three-alternative forced choice test (3-AFC), and flavour profile analysis (FPA) for the detection of taints, off-flavours, flavour discrimination and characterisation.[8,9] Unfortunately, subjectiveness in sensory analysis is particularly acute in flavour perception, since semantic misconceptions regarding flavour are common and data are prone to a great deal of variation from test-to-test and panel-to-panel (typically ± 30–40% or more from one test to the next).[10]

Neither sensory nor instrumental approaches to flavour analysis, in isolation, will provide a complete picture of flavour quality. Motivated by overcoming the limitations of these approaches, combined with the increased distribution and affordability of computing resources, there has been a recent trend towards combined flavour analyses. Combined (instrumental and sensory) analyses consider each approach in combination, by attempting to correlate instrumental data to sensory properties through the use of chemometric techniques such as pattern recognition (PARC), and multivariate analysis (MVA). By taking a combined approach it has been possible to account for some of the sensory properties of many products in terms of the chemical inventory generated by GC-based instrumental data. While such combined analyses have shown promise in being able to correlate sensory and instrument-based data, these are often expensive and time-consuming, requiring sensory panel and instrumental evaluations to be carried out simultaneously as well as involved statistical calculations. We propose that the elements comprising an electronic nose make it well placed to conveniently carry out such combined flavour analysis. For a more thorough discussion of the role of the electronic nose in flavour analysis see ref. 11.

There have been very few studies reporting on generating organoleptic flavour descriptors from chemically sensitive sensor-arrays.[12,13] These studies have focused upon attempts to correlate the data obtained from sensor arrays with heuristic sensory descriptors, as opposed to flavour notes defined under a standardised terminology system underpinned by published reference compounds. We believe that for such an approach to be effective, a generalised method needs to be developed for mapping the non-linear responses obtained from chemical sensor arrays onto such non-linear organoleptic scores. We describe in the next section an odour mapping scheme to be generally applied to the responses obtained from chemically active multi-sensor arrays to obtain a more detailed characterisation of odour quality.

Odour mapping

When using instrumental methods for testing odours, some scheme for the representation of odour quality and intensity (*i.e.*, a coding strategy used for communicating and recording odour) must be made in order to disseminate the data. Current electronic nose systems typically adopt a 1-of-*k* odour representation scheme, where one class of odour is indicated at the exclusion of all other classes. Such analyses of odours have limited application to commerce and industry since they provide only a comparative description of the odour under investigation rather than against an absolute standard. While such a simple odour representation scheme may be useful for discriminating between different complex odours or detecting the presence or absence of food taints (when calibrated against some control), it is not capable of generating any further information about the odorant, in neither chemical nor sensory terms. We believe odour sensing instruments will achieve more widespread application when these are able to carry out detailed and reliable characterisation of simple and complex odours in organoleptic, that is, sensory terms.

The ideal system for representing odour quality would be through the set of "mono-osmatic" or primary odours, that might naturally arise from a unified theory of olfaction. If it were to exist, such a system of primary odours could then be applied universally to all odour stimuli (analogous to chroma, value and hue/saturation in colour description). Unfortunately since the development of such a universal odour representation scheme would rely upon a unified theory of olfaction, a successful scheme looks unlikely to appear in the near future. In view of this lack of known sensory primitives in olfaction, we are limited to describe odours *via* comparisons with other prototypical, and often ambiguous odours (for example by using descriptions such as yeasty, floral, grassy, hoppy).

The development of product specific flavour terminology systems makes use of comparison to develop a set of rigorously defined terminology underpinned by readily available reference compounds. For example, one of the most sophisticated standards systems is used in the brewing industry, devised by the American Society of Brewing Chemists (ASBC).[14] Known as the international flavour terminology system for beer, it is illustrated by the flavour wheel shown in Fig. 1. The system comprises 14 classes, 44 first-tier terms and 78 second-tier terms (not shown) that span the sensory modalities of olfaction, gustation, trigeminal (irritant), and tactile receptors in the mouth. The terminology system is designed such that each separately identifiable flavour characteristic has its own name and there are no terms duplicated. Most importantly, as far as possible the meaning of each term is illustrated with readily available flavour standards, avoiding judgmental terms such as good/bad, young/mature, balanced/unbalanced.[14] Terminology systems such as these standardise the communication of sensory flavour information used in a particular industry, and are useful in training and testing of members of sensory panels as well as in conducting descriptive flavour analysis such as FPA. Training of tasters is carried out through the demonstration of individual reference compounds within standard beer which consequently increases their sensitivity to these. Flavour terminology is also crucial to conducting FPA, whereby the perceived intensity of individual flavour notes (under a suitable terminology system) present in the product are scored by panelists (usually on a scale of 0 to 10, where 0 corresponds to "not present" and 10 to "very strong").

A flavour terminology system makes an excellent choice as an odour representation scheme for an electronic nose for a variety of reasons. Firstly, reference compounds that act as standards to demonstrate particular flavour notes can be used to calibrate an electronic nose for a particular application. Secondly, since these systems are designed to have as far as possible, independent (non-overlapping) terms they easily form the basis of an orthonormal Euclidean odour space within which to represent numerically odour quality. Finally, the numerical scoring usually adopted for use with flavour terminology systems naturally supports the application of statistical and other data processing methods used within electronic nose systems.

The adoption of this method of odour representation, while perhaps not universal in its scope, provides more detailed information relating to the stimulus than currently used odour representation methods in sensor-based machine olfaction. This method is presented as a general technique to be adopted in machine olfaction that supports a more detailed and reliable characterisation of odour stimuli.

The way in which multi-sensor array data produced by an electronic nose might be correlated with FPA data produced by a sensory panel is shown in Fig. 2. Here MVA and PARC techniques may be used to consider the correlation between multi-sensor array data, $\boldsymbol{V}$, and FPA producing the organoleptic data $\boldsymbol{Y}$. The matrix $\boldsymbol{Y}$ comprises n repeated sensory trials of flavour samples, that are scored against p standard flavour terms derived from the flavour terminology system appropriate to the sample. This yields a vector, $(y_{i1}, \ldots y_{ik}, \ldots y_{ip})$, for each sensory trial, i. The matrix $\boldsymbol{V}$ comprises n measurements of the same flavour samples using an electronic nose, where each measurement, i, yields a vector $(\upsilon_{i1}, \ldots, \upsilon_{ik}, \ldots, \upsilon_{iR})$ which represents the pre-processed response from an R sensor array.

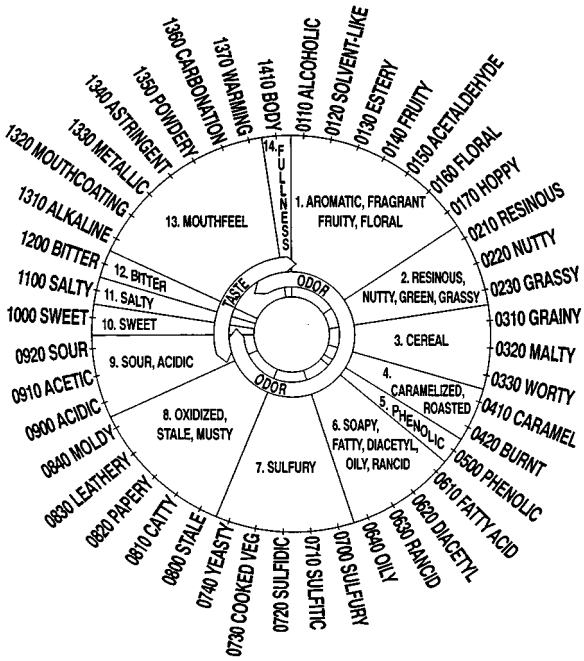

Fig. 1 Flavour wheel used to illustrate the international flavour terminology system for beer. The system was developed over a five year period, jointly by the European Brewery Convention (EBC), the Master Brewers Association of the Americas (MBAA) and the American Society of Brewing Chemists (ASBC) as a means of standardising the terms used in descriptive analysis of beer, as well as for instructing and training sensory panel members. The system consists of 14 classes and 122 separately identifiable flavour terms. Only those terms carrying a four-digit number are intended for use as descriptors and only 40 or so are common in most beers. The remaining flavour notes correspond to flavour-faults or are characteristic of speciality beers. (Reproduced from ASBC, Methods of Analysis[14]).

For an unknown sample j, the task is then to predict a set of organoleptic flavour descriptors $(y_{j1}, \ldots, y_{jk}, \ldots, y_{jp})$, given the array response vector $(\upsilon_{j1}, \ldots, \upsilon_{jk}, \ldots, \upsilon_{jR})$, and a knowledge base of previous measurements $\boldsymbol{Y}$ and $\boldsymbol{V}$. This becomes a mapping problem, with the function $\boldsymbol{V} = f(\boldsymbol{Y})$ being highly non-linear and underdetermined when $R < p$. Associative and mapping neural networks, such as the multi-layer perceptron (MLP), are capable of approximating a solution after training on $\boldsymbol{V}$ with up to n training vectors and targeting on $\boldsymbol{Y}$ with up to n target output vectors.[15] These networks have the advantage of being able to deal easily with the non-linearity of the inverse mapping function, $f^{-1}_{(\bullet)}$ provided that a differentiable, bounded, and non-linear activation function is used for each of the processing elements, and sufficient training data are available. However, it is often difficult to assign a significance level to the outputs from such networks, as can always be obtained from parametric statistical techniques, although the use of activation functions such as "softmax" are part of an ongoing research initiative to provide a more probabilistic interpretation of neural network performance.[16]

An alternative approach is to view the odour mapping process as one of multivariate calibration, to which have been applied a variety of well known chemometric techniques, such as multiple least squares (MLR), partial least squares (PLS), principal component regression (PCR), and canonical correlation analysis (CCA). Such parametric techniques rely on a linear causal model for our mapping function, $f_{(\bullet)}$. For example, MLR uses the following model as its basis

$$\boldsymbol{V} = \boldsymbol{YC} + \boldsymbol{\varepsilon} \tag{3}$$

where $\boldsymbol{C}$ are the linear regression terms ($p \times R$ matrix) that relate the organoleptic flavour scores, $\boldsymbol{Y}$, to the pre-processed sensor responses, $\boldsymbol{V}$. The residuals for the regression, $\boldsymbol{\varepsilon}$ ($n \times R$ matrix), represent the errors for the best linear fit, having an expectation of zero. Given the data for $\boldsymbol{V}$ and $\boldsymbol{Y}$, it is possible to solve for $\boldsymbol{C}$ and then $\boldsymbol{\varepsilon}$, using the generalised inverse method

$$\boldsymbol{C} = (\boldsymbol{Y}^{\mathrm{T}}\boldsymbol{Y})^{-1}(\boldsymbol{Y}^{\mathrm{T}}\boldsymbol{V}) \tag{4a}$$

$$\boldsymbol{\varepsilon} = (\boldsymbol{V} - \boldsymbol{YC})^{\mathrm{T}}(\boldsymbol{V} - \boldsymbol{YC}) \tag{4b}$$

for any dimensionality of $\boldsymbol{C}$, provided that the inverse matrix exists. If $R < p$ then the system of equations in eqn. (3) is underdetermined and an infinite number of solutions exist. However, Denham and Brown discuss methods that can be applied to resolve this indeterminacy.[17] Having estimated $\boldsymbol{C}$ it is then possible to make the required prediction of the organoleptic scores, $\hat{\boldsymbol{Y}}$, for unknown sensor responses, $\hat{\boldsymbol{V}}$, using

$$\hat{\boldsymbol{Y}} = \hat{\boldsymbol{V}}\boldsymbol{C}^{-1} \qquad (R = p) \tag{5a}$$

$$\hat{\boldsymbol{Y}} = \hat{\boldsymbol{V}}\boldsymbol{C}^{\mathrm{T}}(\boldsymbol{C}\boldsymbol{C}^{\mathrm{T}})^{-1} \qquad (R > p) \tag{5b}$$

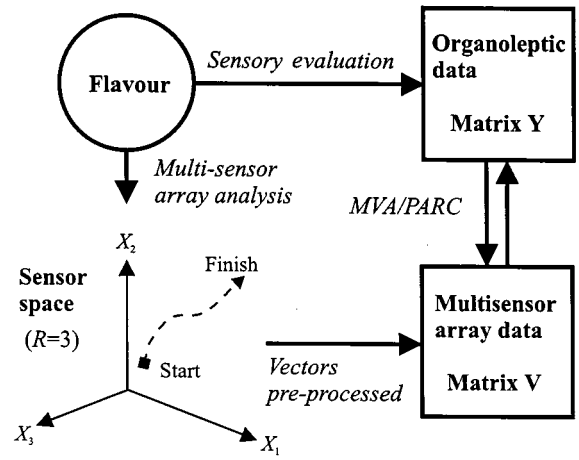

Fig. 2 Correlating organoleptic data as produced by a sensory panel with the response of a multi-sensor odour array.

The use of MLR in this context also assumes that each of the organoleptic scores and sensor responses within $\boldsymbol{Y}$, $\hat{\boldsymbol{V}}$ and $\boldsymbol{V}$, respectively, are truly independent. If $\boldsymbol{Y}$, $\hat{\boldsymbol{V}}$ and $\boldsymbol{V}$ are not of full rank, then at least one of the columns is a linear combination of the others within the same matrix. While the design of flavour terminology systems attempts to make terms as independent as possible, there is no guarantee that all subsets of flavour scores will not be collinear. Also most chemical sensors have overlapping sensitivities to analytes leading to collinearity in their response. For this reason the use of PLS which forces the latent variables to be orthogonal and so does not suffer from the collinearity problem is likely to be of more general use in odour mapping. Methods such as PLS and PCR are also less sensitive to noise and make no requirement regarding the relative dimensionality of the data.

The linearity assumption of these parametric techniques can be overcome by linearising the chemical-sensor and organoleptic responses beforehand. Typically the concentration dependence of many chemical sensors can be approximated as linear for low concentrations of analyte. For example, by considering the response of conducting polymer films to be governed by a Langmuir adsorption process, Gardner *et al*. provide a steady-state conductance model, where the fractional change in conductance is given by[18]

$$\frac{G_{t=\infty} - G_{t=0}}{G_{t=0}} = -S\frac{bC}{(1+bC)} \qquad (6)$$

and where ($b = k_a/k_d$) is the active site affinity constant, C is the analyte concentration, $G_{t=\infty}$ is the steady-state conductance, $G_{t=0}$ is the baseline conductance in the absence of the analyte, and S is an analyte sensitive constant. For low concentrations ($bC \ll 1$), the fractional change in conductance is approximately linear with analyte concentration. For higher concentrations, sensor response may be linearised using eqn. (6).

The intensity or sensory-impact for a specific flavour notes has been modelled by Steven's power law of psychophysics[19]

$$R = c\,(C - C_0)^n \qquad (7)$$

where R represents the flavour intensity as reported by the perceiver and c is a constant of proportionality that is specific to the individual, and n is an index that varies between 0.2–0.8 depending upon the odorant in question. Consequently, up to the terminal threshold (the concentration beyond which no further increase in intensity is reported), perceived flavour note scores behave log-linearly with concentration and so linearisation for each note within a particular terminology system is possible. By linearising both the chemical-sensor and organoleptic responses, linear chemometric techniques can be directly applied to provide an odour mapping that will also yield statistical measures of performance. However, in practice these techniques are often outperformed by associative and mapping neural networks due to their ability to deal with generalised non-linearity within the data-set, but often at the loss of rigorous performance metrics.[20]

Sensor optimisation

The substrates of the conducting polymer resistive odour sensors were fabricated using microtechnology in a process developed over a considerable period of time. The photolithography process was made compatible with an initial cleaning of the gold micro-electrodes by cycling the potential in 2 M sulfuric acid, followed by the electrochemical deposition of the conducting polymers in either aqueous or organic solutions. The fabrication process for early sensors used wafers made of alumina rather than silicon, and is already published by Pearce *et al*.[21] The electrochemistry and characterisation of the conducting polymers took place at Southampton University and have also been reported.[22] These studies focused on identifying the geometrical factors that contribute to the resistance of the devices and a homogeneous model was produced which gave a good fit to the experimental data.[18] An electrode gap of *ca*. 10 μm and a film thickness of *ca*. 1 μm were found to be practical values to adopt—both in terms of yielding negligible geometric process error (in a 1.0 μm process) and reasonable values of the response time and device resistance. More recently, the fabrication process has been moved to silicon-based microtechnology to be consistent with the design and fabrication of resistive sensors employing semiconducting oxide films.[23] In a related study, a full investigation of silicon-based conducting polymer odour sensors has been carried out and has shown that, although the sensor responses to odours were broadly similar to alumina-based devices, the batch-to-batch reproducibility was enhanced with the device variation being reduced from about 30% to around 10% or better.[24]

In this study, our chosen electrode geometry and growth conditions set the device resistance to lie in the range of 100 to 10 kΩ which was desirable for input resistance to the interface electronics. The lead and connection resistances were thus comparatively small because we used short leads and high-quality, gold-plated headers. It was observed that the polymer sensors "drifted" when a voltage of 1 V was applied across the electrodes, which we attributed to protonation within the electro-active polymer film over time. Consequently, the maximum voltage drop across the polymer within a constant-current circuit was set to ±100 mV. The low bias voltage not only reduced this effect but also ensured that the $I(V)$ characteristic was reasonably Ohmic for all of our polymer films. Furthermore, this low voltage also reduced the effect of field-induced drift which can occur when there is a high concentration of the analyte that solvates and hence immobilises ions within the film. The problem was particularly marked for some polymers when CO_2 was used as the carrier gas and so all experiments employed nitrogen in which the polymers appeared to be stable.

The effect of the temperature upon the resistance of conducting polymers has already been reported and typically gives a large temperature coefficient of up to *ca*. 1×10^{-2}/°C.[25,26] The effect of temperature on both the baseline resistance and response was minimised here through maintaining the sensor chamber at (30 ± 0.1) °C, *i.e.*, to produce a relative error of less than 0.1%. Finally, it is well known that the resistance of certain conducting polymers depends strongly on water vapour (*e.g.*, polyanilines). Recent work indicates that the effect of water can be described by a competitive binding model and thus has a predictable and reversible effect on the sensor response.[27] The effect of changing water vapour was minimised in our experimental work through its control in a fully automated FIA system, and the use of a closed system isolating the samples from diurnal changes in ambient conditions. Despite all of these precautions, there still appeared to be a long-term drift that was significant when attempting to resolve sub-ppm taints in samples. This was attributed to a general poisoning of the polymer because of the nature of the headspace of lager beers. Its effect was to give a gradual decrease in the base-line resistance of the polymers over the testing period rather than reduce the magnitude of the response to the analyte. Thus we feel that the problem can be addressed by an appropriate choice of signal processing, perhaps in a similar way to adaptation in the human olfactory system.[28]

Experimental

Flow injection analyser

We have previously described a simple instrument for the evaluation of odours using a conducting polymer array

combined with a manual headspace injection system.[21] A more sophisticated FIA has also been developed, providing superior control over the amount of analyte being introduced into the sensor head and operating temperature. The automated operation of the analyser also minimises human intervention in the testing process and thereby reduces experimental error to obtain more reproducible data.

This system combines chemical and electronic apparatus to provide microcomputer control of the sampling sequence. The sampling procedure is software configurable *via* the interfacing circuitry shown. Concomitantly, sensor data are acquired from the rig to provide a real-time system for rig monitoring and control. A schematic of the odour-sampling system is shown in Fig. 3, where chemical hardware components are shown as BS 2917/ISO 1219 symbols. The system allows up to three separate samples to be loaded at any one time into the sample vessels shown. Two software configurable quad normally closed (NC) manifold valves (supplied by Neptune Research, model no. 225T07) are used either side of the sample vessels to route flow to any combination of these elements shown in the centre. Flow into the rig is controlled *via* 3 mass flow controllers (MFCs), shown on the left of Fig. 3. These MFCs (supplied by Brooks Instrument BV, model no. 5850 TR) are fully programmable and incorporate a flow-rate control valve, an override stop valve and a flow-rate meter for system feedback and are used to control flow independently from 3 pressurised gas sources within a 0–5 ml s^{-1} range to within an accuracy of 1% FSO. The PTFE tubing used throughout has an internal diameter of 2.41 mm and permits a maximum flow velocity of 0.912 cm s^{-1}.

The three carrier gases used here are nitrogen in line 1, air in line 2 (both wet), and dry carbon dioxide in line 3, scrubbed by particulate matter filters (which trap particles with diameter greater than 35 μm, supplied by Lee Products Ltd., part no. TCFA1201035A). A non-return valve is also used after each MFC to prevent pressure differentials causing backwards flow within the system. A 5-way 0.157 in Boss manifold (supplied by Lee Products, part no. TMMA 9500190Z) acts as a mixing chamber at the juncture of the three flows in order to combine the carrier gases. Hermetically sealed Dreschel bottles (250 ml) and head fittings are used as sample vessels with the carrier gas made to percolate through the liquid sample. Glassware and in-line elements after the sample vessels are maintained at a constant temperature through the use of heating tape (supplied by Eurotherm) to prevent condensation of vapour and thereby causing interference. Wherever possible, Teflon components have been used as these are inert and avoid significant odour retention. A miniature two-way solenoid valve (supplied by Lee Products, part no. LFAA12001L8H) provides a sensor head bypass to prevent forwards flow interfering with the prevailing conditions in the sensor head, which is used during cleaning. A cleaning bypass is also provided to purge the associated tubing and elements free from contamination after each individual sampling phase without interfering with the samples. The purging bypass is used to allow the sensor head to be prepared in an atmosphere of fresh carrier gas prior to sampling.

The sensor head is capable of housing up to 24 conducting polymer resistive sensors, mounted on a conical block which promotes laminar flow across the sensors. The temperature within the sensor head can be controlled independently from the rest of the test-rig through the use of a heating mat, thus allowing temperature effects within the sensors to be investigated easily. A platinum resistance thermometer and a capacitive polymer humidity sensor (supplied by Lee-Integer, part no. ACH-1000) are mounted within the sensor head in order to measure temperature and humidity to an accuracy of ±0.1 °C and ±1% RH, respectively. A number of factors were considered in the design of the FIA in order to minimise the possibility of contamination. In particular, inert materials were used for components after the sample vessels, and the flow path to the sensor head was minimised.

The electronic hardware for the flow injection analyser has been incorporated into a standard KM6 Eurorack (supplied by BICC-VERO Electronics) in Eurocard formfactor modules, compliant with DIN 41494 part 5, IEC 297 section 3 and IEC subcommittee SC 48D. The overall schematic diagram for the rack-system, complete with backplane and external connectivity is shown in Fig. 4. The interfacing to the 24 conducting polymer sensors within the sensor head is provided by four cards each providing for six sensors (Module 2) as already described in ref. 21. Temperature and humidity sensor interfacing as well as driver circuitry for the chemical hardware is provided by two separate Eurocards (Module 3 and Module 5, respectively). Two system cards (Module 4) are necessary to mediate between the PC-based data acquisition cards (supplied by Data Translation, model no. DT-2811 PGH) and the rack system. These cards also synchronise the data acquisition duty cycle for the system as a whole.

The custom-designed software of the system provides facilities for batch data acquisition, a real-time rig monitor, data storage, sensor array calibration, data pre-processing, data plotting, rig status reporting, and a chemometric fingerprinting technique described previously.[21] A comprehensive software controlled batch processing system provides a fully automated sampling sequence with adjustable testing parameters. The batch testing process is based upon the sequence shown in Fig. 5. Testing begins with a pre-clean phase necessary to ensure that no contamination has built up within the rig since last being used. This phase involves passing a mixture of the three carrier

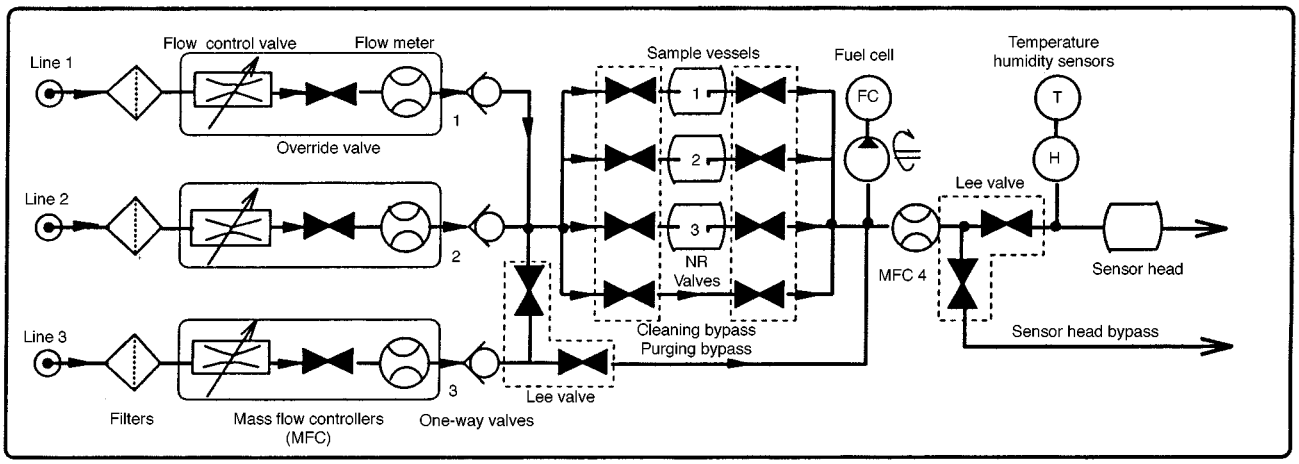

Fig. 3 Chemical hardware for the flow injection analyser, showing from left to right: gas bottle sources (lines 1, 2 and 3); line filters; MFCs; non-return valves; two way purging valve; quad normally closed (NC) valves; sample vessels; fuel cell with compressor pump; final flow meter; sensor head bypass valve; and sensor head: housing temperature; humidity and odour sensors.

gases (N_2, air, and CO_2) at a high flow-rate through the clean sample vessels and through to the sensor head bypass. This drives impurities from within the glassware, associated piping and control elements out through the exhaust. A comprehensive set of diagnostic tests are then carried out on the rig to ensure correct operation giving better confidence in the data.

An operator is only required to load and unload samples. During loading, samples pre-heated to 30 °C in a separate temperature controlled water bath are inserted into the sample vessels which are then hermetically sealed. During unloading, these sample vessels are then removed and replaced by empty, analytically clean vessels. Immediately after the samples have been loaded, a set-up phase injects a full headspace of operator selectable carrier gas into each sample vessel. Then the quad manifold valves are automatically closed to seal the vessels, providing a fresh headspace to which the odorous sample equilibrates.

The main testing cycle can now begin, iterating for each individual sample. With the sample vessels still closed off, the rest of the rig is cleansed by passing an operator-selectable carrier gas through the cleaning bypass and out through the sensor head bypass. This forms the main cleaning phase between sampling phases. Prior to sampling proper, a purging phase establishes a gaseous environment within the sensor head. This provides a reference environment and allows the sensors to settle to their respective baseline values. Sampling begins by selecting the quad valves to the appropriate sample vessel and commencing the pre-set flow profile through the sample vessel. The odorous headspace, established within the sample vessel during the previous cleaning and purging phases, is now driven along to the sensor head and over the sensor array at the initial flow-rate specified in the flow profile. Data are logged from each sensor. The flow profile used during sampling is fully adjustable. After unloading, a post-clean phase, identical to the pre-clean phase, is carried out to remove any remaining contaminants from the last sampling phase.

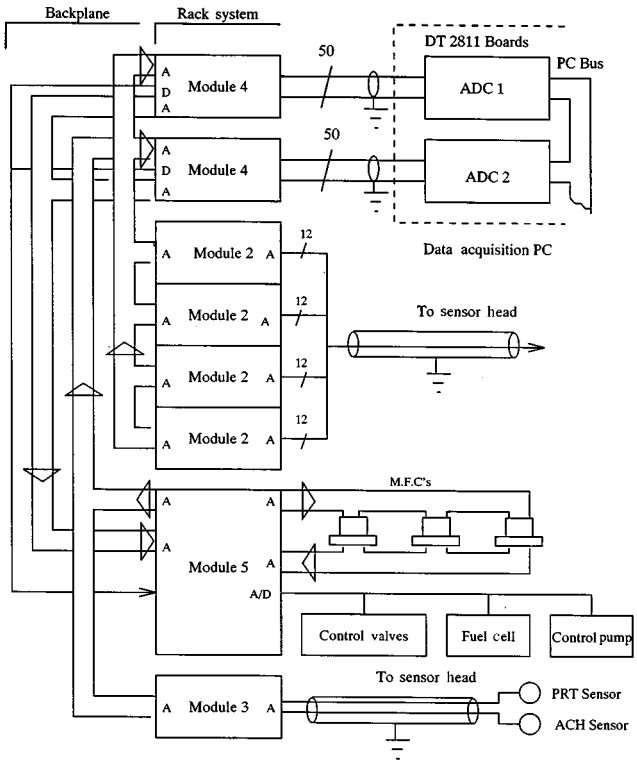

Fig. 4 Schematic of electronic hardware for the flow injection analyser: Showing Module 2, 6 channel conducting polymer interface card; Module 3, temperature and humidity interface cards; Module 4, system cards; Module 5, flow injection rig controller. (A) - analogue signal (D) - digital signal.

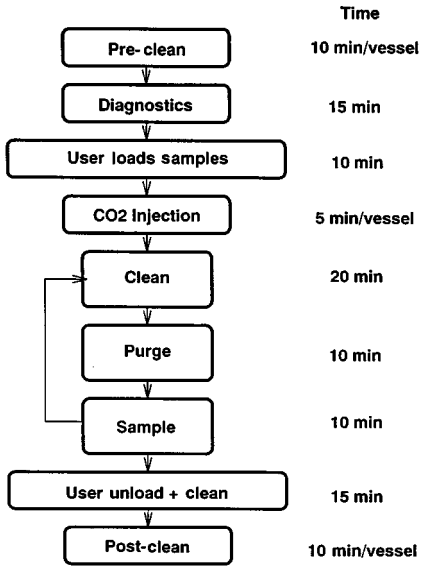

Fig. 5 Flow-chart of the sampling sequence complete with optimised phase durations.

Experimental design

The ability of this FIA system to identify individual reference compounds has already been reported by the authors in a preliminary study.[29] This work demonstrated the ability to discriminate diacetyl taint at the sub-ppm level in a background of 4% v/v ethanol solution from control ethanol solution, using the FIA system described above combined with an array of 18 conducting polymer devices and an MLP neural classifier.

An experiment was now designed to assess the application of the odour mapping technique described earlier to multi-sensor arrays for identifying individual flavour notes within a chemically complex odour background (in this case lager beer). The particular flavour notes investigated from the ASBC flavour wheel are summarised in Table 1. In all, three reference compounds were added to the control lager beer, Bass Carling Black Label (CBL) at varying concentrations—2,3-butanedione

Table 1 Flavour spikes added to standard Bass Carling Black Label lager (4% alc. v/v) prepared for FIA study. Table shows the reference compounds for three flavour notes chosen from the ASBC (International Flavour Wheel for Beer). The spike concentrations can be compared against the typical sensory thresholds for these compounds in humans. The corresponding organoleptic (panel) score results from a sensory panel conducting FPA are given

Flavour spike	Sensory threshold/ ppm	Spike conc./ ppm	Flavour note	Panel score (1–10)
2,3-Butanedione	0.07	0.1	No. 0620 Diacetyl	1
2,3-Butanedione	0.07	0.2	No. 0620 Diacetyl	2
2,3-Butanedione	0.07	0.3	No. 0620 Diacetyl	4–5
2,3-Butanedione	0.07	0.4	No. 0620 Diacetyl	6–7
Hop essence	80	100	No. 0170 Hoppy	1
Hop essence	80	200	No. 0170 Hoppy	2
Hop essence	80	300	No. 0170 Hoppy	5–6
Hop essence	80	400	No. 0170 Hoppy	7–8
Dimethyl sulfide	30 ppb	20 ppb	No. 0730 Cooked veg.	0
Dimethyl sulfide	30 ppb	40 ppb	No. 0730 Cooked veg.	1
Dimethyl sulfide	30 ppb	60 ppb	No. 0730 Cooked veg.	3
Dimethyl sulfide	30 ppb	80 ppb	No. 0730 Cooked veg.	5–6

leading to the butterscotch off note or 'diacetyl' note (ASBC note no. 0620), hop essence leading to the "hoppy" note (ASBC note no. 0170), and dimethyl sulfide leading to the "cooked vegetable" note (ASBC note no. 0730). The sensory thresholds shown in Table 1 represent the concentrations at which the spikes become flavour active to most sensory-panel members, given the known background of CBL. All of these reference compounds are typically present in beers between 0.5–2 times the average sensory threshold, which are classified as "secondary" constituents. Flavour components within this category are known to account for differences between beers of the same type and are the most vital from a quality assurance perspective, which motivated their use in this study.

The concentration ranges of the reference compounds were chosen to coincide with the natural organoleptic ranges for each of the flavour notes. The scores for each note (between 0–10) were estimated on an empirical basis, using known sensitivities to these compounds in sensory panel members to CBL.[30] Another factor motivating the selection of flavour notes was the availability of published reference compounds, and a knowledge of the flavour-impact of these compounds within the context of the control lager. As such, these scores represent what may typically be reported by a trained sensory-panel conducting FPA on the samples (although these values will typically vary by 30–40% over time). As can be seen from Table 1 these scores typically behave log-linearly with spike concentration, as predicted by Steven's power law of psychophysics, eqn. (7).

Samples were supplied in 2 l bottles that have a limited shelf-life of two weeks. This restricted the number of samples that could be taken within a single batch. Given the 40 min sampling time required per sample, and pre- and post-cleaning times as shown in the sampling sequence, Fig. 5, it was possible to process up to eight samples a day or roughly 80 samples in a two week period. Therefore six samples of each class (13 classes) were taken, making 78 samples in all. The samples from each class were drawn randomly using a latin square design to reduce any experimental bias in the data.

The experimental conditions used for the preliminary testing were adhered to, although a number of notable alterations were required. Firstly, the temperatures for both the sample vessels and sensor head were reduced to 25 and 30 °C, respectively. This was done to reduce further the possibility of oxidation of the beer samples, which may seriously alter the headspace odour during sampling. An odourless anti-foaming agent (supplied by Bass Breweries research laboratories) was also added to each sample to prevent foaming during testing, which would enter piping and the sensor head to disastrous effect. The sensor array shown in Table 2 was used to provide an increased variety of sensor types for the study. As in the preliminary study, two devices of each type were selected in order to check the reliability of sensor response. The initial baseline resistances for each sensor, as measured before the start of testing, are also given, some of which showed considerable variation for identical polymers, with an average value of 10–30% being observed.

Rig optimisation and commissioning

Before testing proper could begin, a number of pilot runs were conducted in order to optimise the testing parameters associated with the FIA rig, as identified in Table 3. The parameters fall into four categories: operator-adjustable; software configurable (but not operator adjustable); manually adjustable; and non-adjustable (variables). The manually adjustable parameters are controlled through the associated chemical hardware. Other non-adjustable variables are a consequence of the nature of the sample (*e.g.*, ethanol concentration within the headspace: variable 11), rig design (*e.g.*, sensor head pressure and sample humidity: variables 12 and 25, respectively), or are dependent upon other parameters (*e.g.*, minimum and maximum equilibrium times: variables 20 and 21, respectively).

In the optimisation of parameters used for testing, the following criteria were considered: (1) Optimise response—the parameter-set should maximise response of the sensor array to the sample whilst minimising test-to-test variation. (2) Minimise contamination—the parameter-set should minimise run-to-run and batch-to-batch contamination. (3) Minimise testing time—phase times should be kept to a minimum in order to maximise sample throughput. (4) Sample considerations—the parameters must provide a suitable environment for the sample, without causing spoiling.

Optimisation of the parameter-set proceeded by considering each parameter type in turn. The manually adjustable parameters were considered to be sample specific, principally being constrained by the type of sample (in this case lager beer). For example, the sample vessel temperature (variable 22) was preset at 25 °C so as not to affect flavour volatiles with the headspace. To avoid contamination of the rig, the piping (parameter 23) and sensor head (parameter 24) were heated to 5 °C higher than the sample temperature, constraining these both to 30 °C.

Using either wet air ($\approx$ 50% RH) or dry CO_2 as the headspace and carrier gas for sampling (parameters 9 and 16) was avoided due to the possibility of oxidation of the beer samples. The use of these carrier gases also appeared to cause instability in the sensor responses, believed to be attributed to immobilised ions within the polymer film causing field-induced drift. Therefore N_2 was chosen as the set-up and carrier gas agent, giving the most repeatable sensor response from test-to-test. The set-up time period (parameter 14) and flow-rates (parameter 15) were set to 5 min per vessel and 4.8 ml s^{-1}, respectively, and were based upon the deadspace and flow-rates within the system to ensure at least one full replacement of the headspace above the

Table 2 FIA sensor array

No.	Sensor type	Initial resistance/Ω	No.	Sensor type	Initial resistance/Ω
1	PPy/TEATS/H_2O	77.1	13	PPy/PSA/H_2O	16.86 k[a]
2	PPy/TEATS/H_2O	403.1	14	PPy/PSA/H_2O	4.96 k
3	PPy/TEATS/PC	231	15	PPy/HpSA/H_2O	162.6
4	PPy/TEATS/PC	347	16	PPy/HpSA/H_2O	154
5	PPy/pTSA/EtOH	99	17	PPy/HxSA/H_2O	693
6	PPy/pTSA/EtOH	67	18	PPy/HxSA/H_2O	302
7	P3MT/TEATFB/CH_3CN	171.5 k	19	PPy/DSA/H_2O	77.1
8	P3MT/TEATFB/CH_3CN	74.8 k	20	PPy/DSA/H_2O	81.1
9	PAN/$NaHSO_4$/H_2O	3.06 k	21	PPy/pTSA/H_2O	30.3
10	PAN/$NaHSO_4$/H_2O	2.70 k	22	PPy/pTSA/H_2O	38.9
11	PPy/BSA/H_2O	203.6	23	PPy/DSA/H_2O	339
12	PPy/BSA/H_2O	460.5	24	PPy/DSA/H_2O	183

[a] Sensor removed during study due to systematic drift causing response beyond ADC scale.

Table 3 Key physical variables and parameters for the FIA rig

No.	Variable	Unit	No.	Variable	Unit
1	Cleaning time[a]	s	14	Set-up time[b]	s
2	Cleaning flow[b]	ml s^{-1}	15	Set-up flow[b]	ml s^{-1}
3	Cleaning agent[a]	—	16	Set-up agent[b]	—
4	Purging time[a]	s	17	Pre/post-clean time[b]	s
5	Purging flow[b]	ml s^{-1}	18	Pre/post-clean flow[b]	ml s^{-1}
6	Purging agent[a]	—	19	Pre/post-clean agent[b]	—
7	Sampling time[a]	s	20	Min equilibrium time	s
8	Sampling profile[a]	ml s^{-1}	21	Max equilibrium time	s
9	Sampling agent(s)[a]	—	22	Sample vessel temp.[c]	°C
10	Sample stirring[c]	—	23	Pipe temperature[c]	°C
11	Headspace EtOH conc.	ppm	24	Sensor head temp.[c]	°C
12	Sample humidity	% RH	25	Sensor head pressure	mbar
13	Data filtering[a]	—	26	Bubbling/non-bubbling[c]	—

[a] Denotes those parameters that are operator-adjustable. [b] Denotes software configurable parameters. [c] Denotes manually adjustable test conditions.

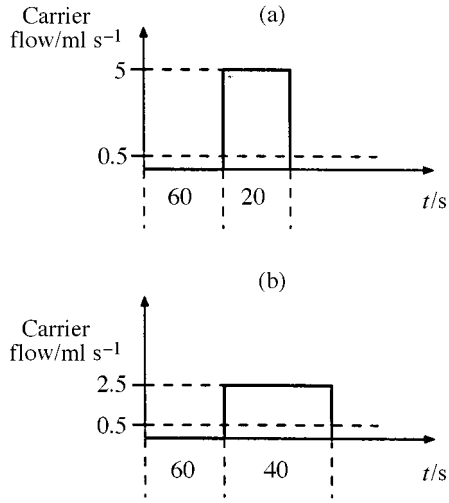

Fig. 6 Two carrier gas flow profiles used to optimise the array response: (a) 5 ml s^{-1} maximum; and (b) 2.5 ml s^{-1} maximum.

samples before sealing. Cleaning (parameter 2) and purging (parameter 5) flow-rates were maximised (4.8 ml s^{-1}), in order to minimise the required cleaning and purging periods. A purging time of 10 min was found to be sufficient to ensure full replacement of the sensor-head deadspace.

The remainder of the operator-adjustable and software configurable parameters were optimised after a series of comparative studies on the same sample (control CBL lager) during commissioning of the FIA system. The two flow profiles (parameter 8) shown in Fig. 6 were used for testing control CBL, in order to maximise the sensor array response. Both flow profiles deliver an initial odour pulse which transfers 100 ml of headspace from the sample vessel directly to the sensor head. Using the profile shown in Fig. 6a this is achieved in 20 s, whereas that shown in Fig. 6b requires 40 s. During comparative testing using these profiles, a similar array response was observed for both, although using the second method of odour delivery required more time for the sensors to reach equilibrium with the sample. Consequently the flow profile shown in Fig. 6a was adopted for all subsequent testing.

Comparative tests were also carried out to observe the effect of using bubbling and non-bubbling carrier gas sample percolation (parameter 26). The magnitude and repeatability of array response favoured the non-bubbling sampling method and so was used throughout testing. In order to isolate the effect of pre/post-cleaning times (parameter 17), flow-rate (parameter 18) and agent (parameter 19) and the effect of cleaning times (parameter 1), flow-rate (parameter 2) and agent (parameter 3), upon possible run-to-run and batch-to-batch contamination within the sampling apparatus, a set of tests were executed. This

Table 4 Test set used to optimise cleaning parameters

Sample Vessel	Test 1	Test 2	Test 3	Test *n*
1	Sample	Blank	Sample	....
2	Blank	Sample	Blank	....
3	Sample	Blank	Sample	....

involved iterating an alternating sequence of full and empty samples in the three vessels, as shown in Table 4, whilst maintaining and monitoring all other variables and parameters as constants.

By examining the response of the sensor array to the blank sample tests under various conditions of pre/post-cleaning and cleaning parameters, it was now possible to assess the efficiency of these phases in cleansing the apparatus. The response of the sensor array during the blank samples of tests 1 and 3 was observed to ensure that sufficient cleaning time had been used to remove any contaminants from previous samples. Likewise, the response of the sensor array during the blank samples of test 2 was observed to ensure that sufficient pre-cleaning and post-cleaning time had been used to remove any contaminants from previous test batches. This process was iterated whilst varying cleaning parameters to ensure that no interference between samples occurred. The final phase times after optimisation are shown alongside the sampling sequence in Fig. 5.

In Part 2 of this paper we describe the computational analysis of the data arising from this experiment and so assess the efficacy of the odour mapping technique described here in generating organoleptic flavour note information.[31]

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