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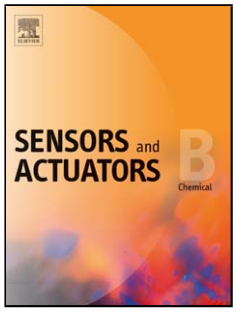

Title: Methodological Variation in headspace analysis of liquid samples using electronic Nose

Authors: Henri Knobloch, Claire Turner, Andrew Spooner, Mark Chambers

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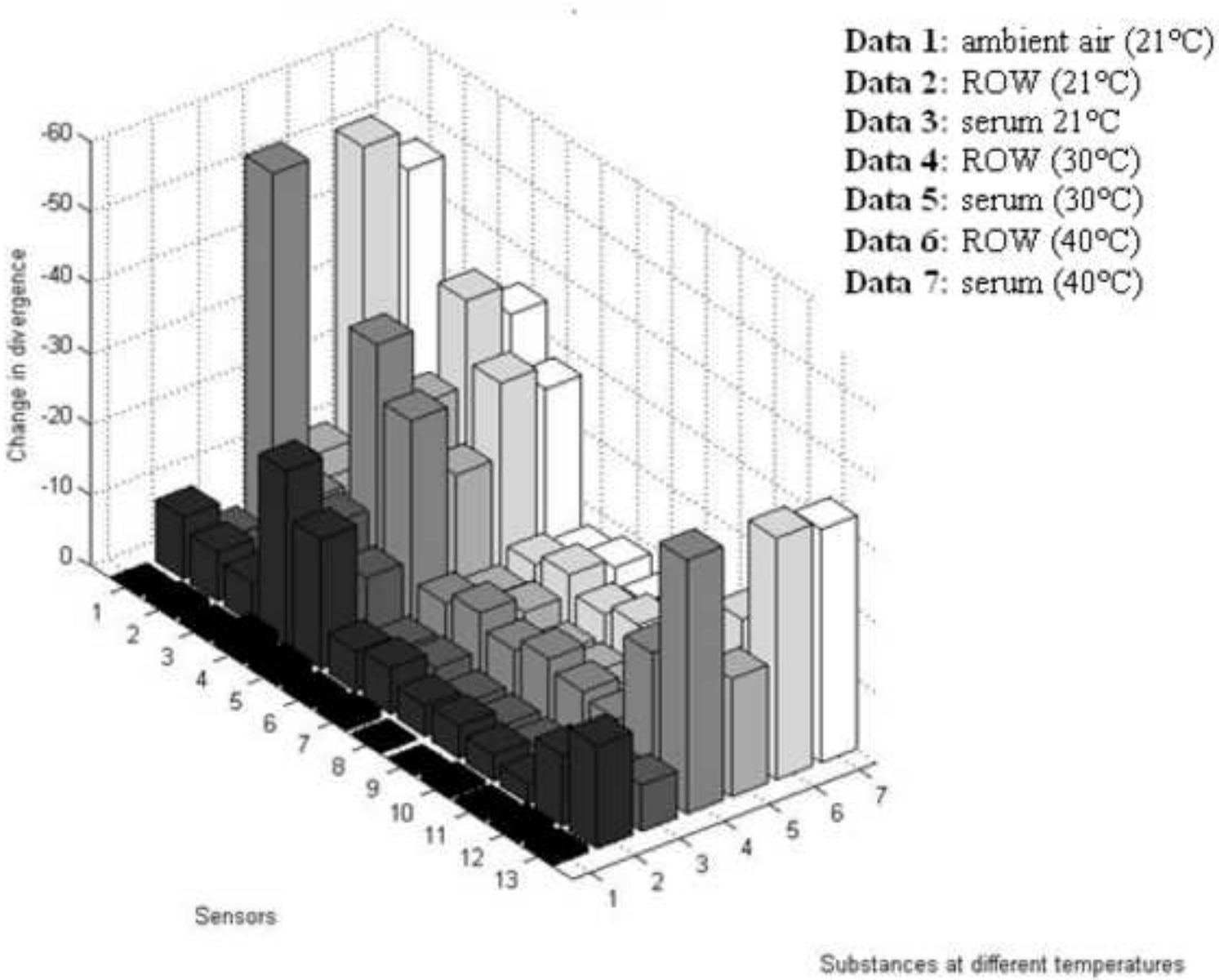
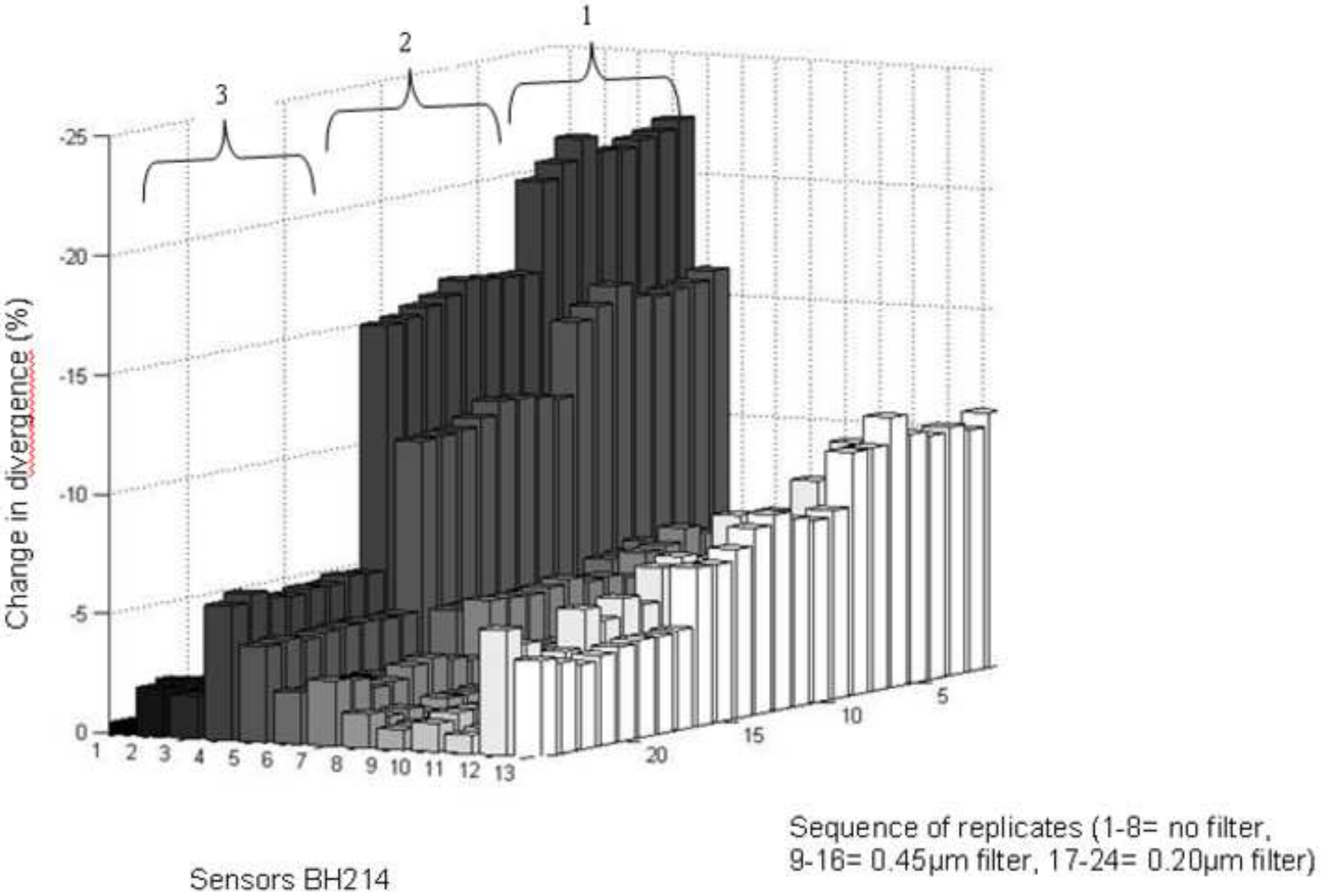
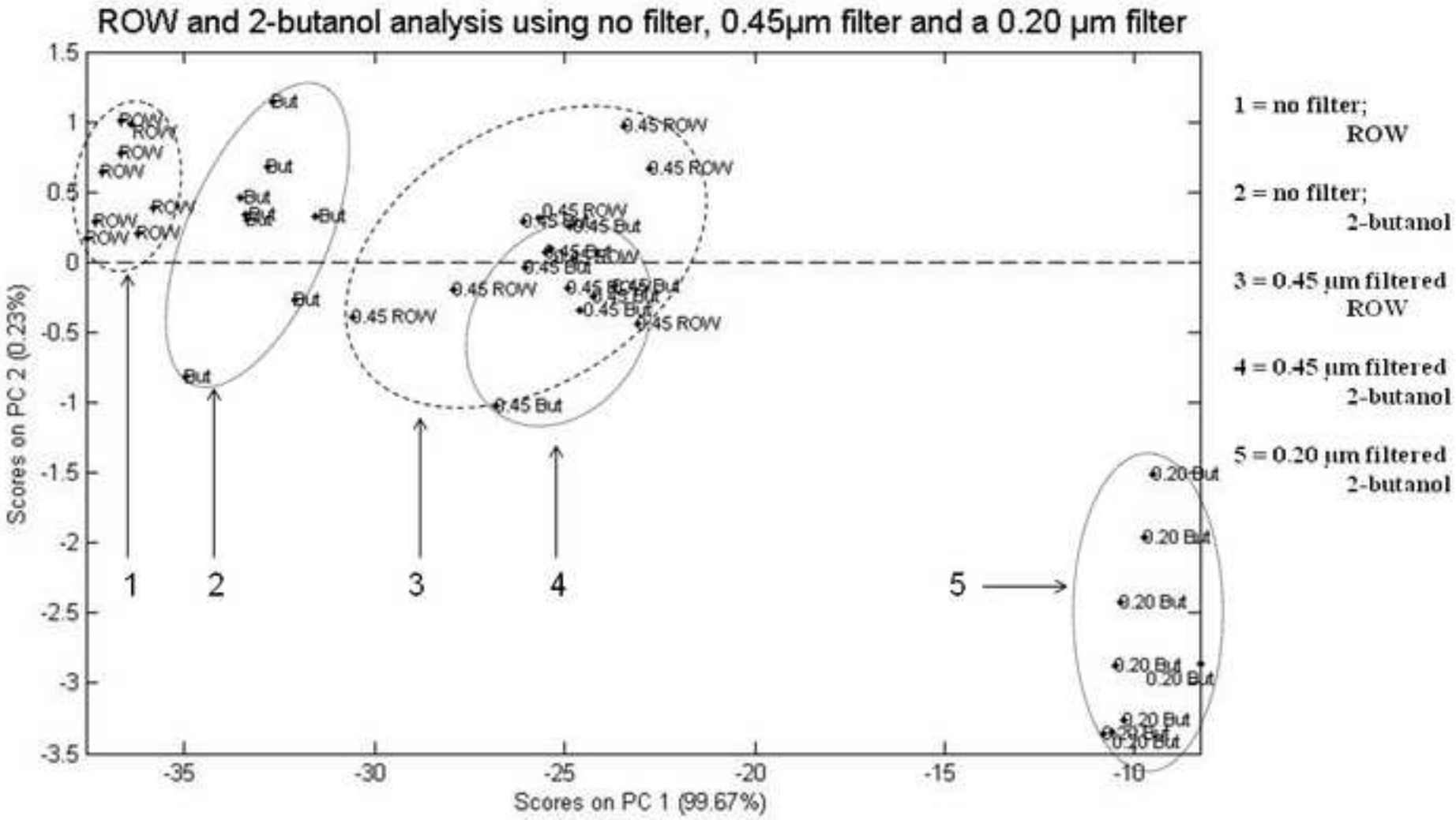
Figure(s)


Data 1: ambient air (21°C)
Data 2: ROW (21°C)
Data 3: serum 21°C
Data 4: ROW (30°C)
Data 5: serum (30°C)
Data 6: ROW (40°C)
Data 7: serum (40°C)
Change in divergence
-60
-50
-40
-30
-20
-10
0
1
2
3
4
5
6
7
8
9
10
11
12
13
Sensors
1
2
3
4
5
6
7
Substances at different temperatures

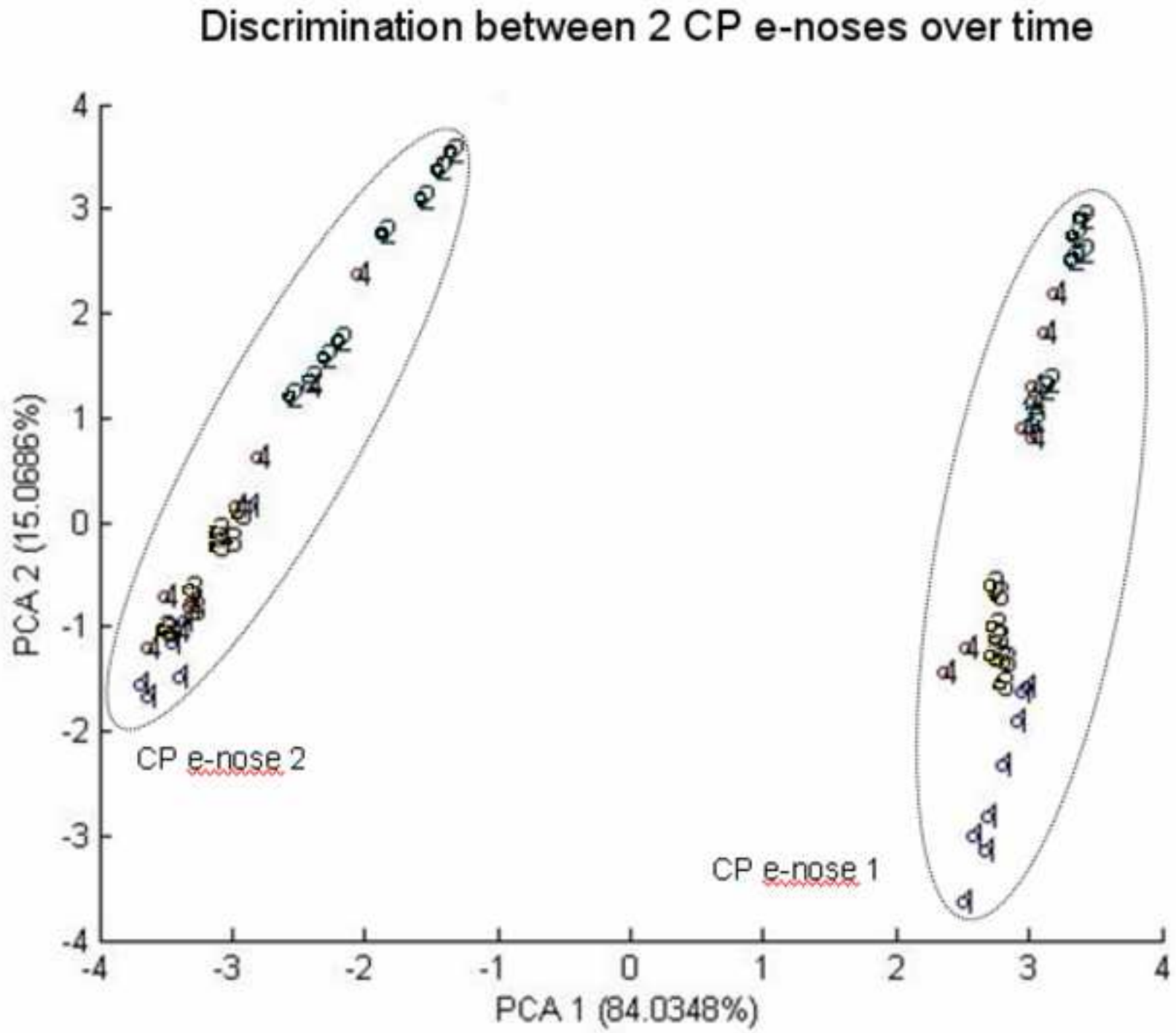
Figure(s)

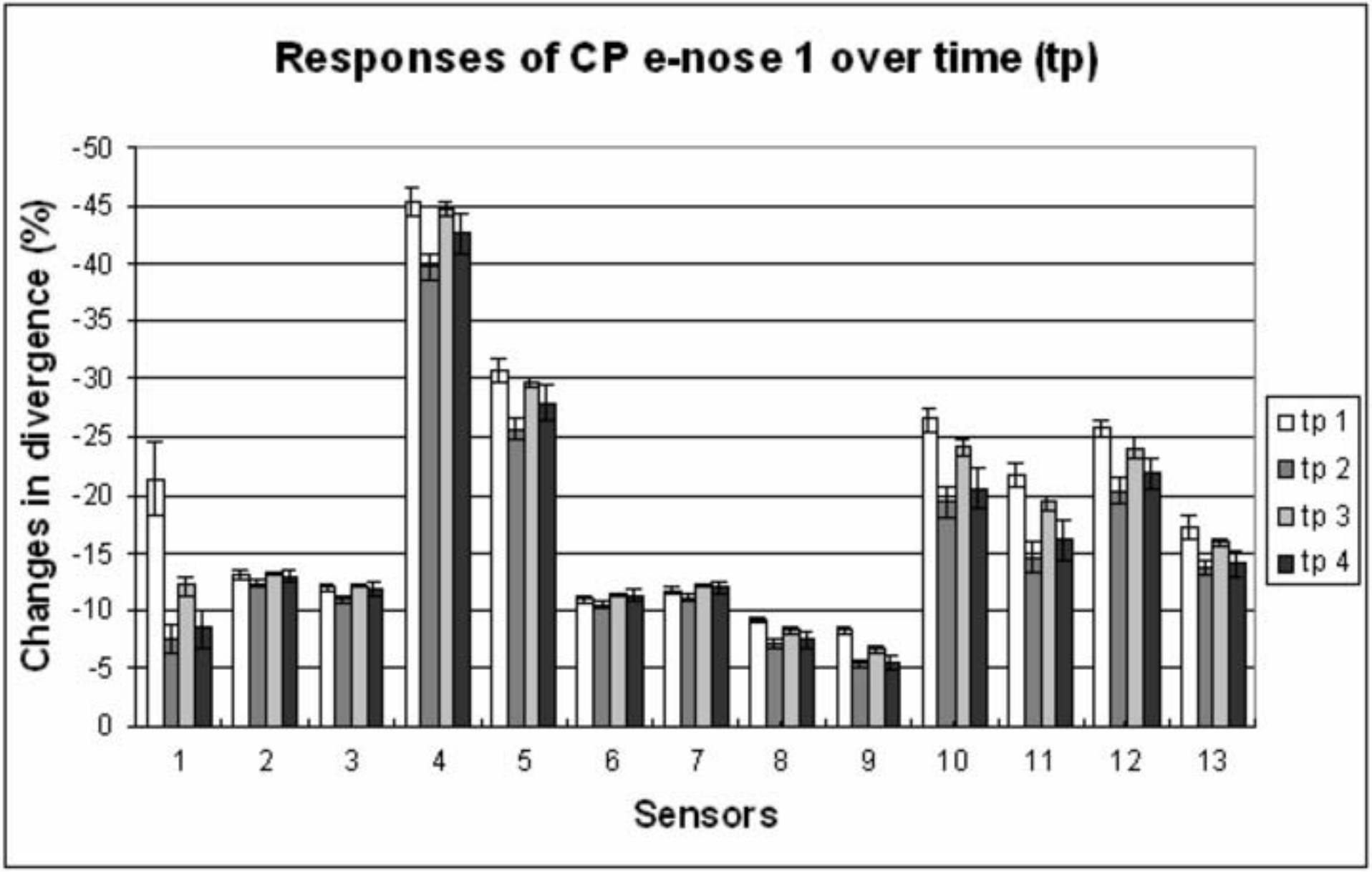

1
2
3
-25
-20
-15
-10
-5
0
Change in divergence (%)
1 2 3 4 5 6 7 8 9 10 11 12 13
20
15
10
5
Sensors BH214
Sequence of replicates (1-8= no filter,
9-16= 0.45µm filter, 17-24= 0.20µm filter)

Figure(s)

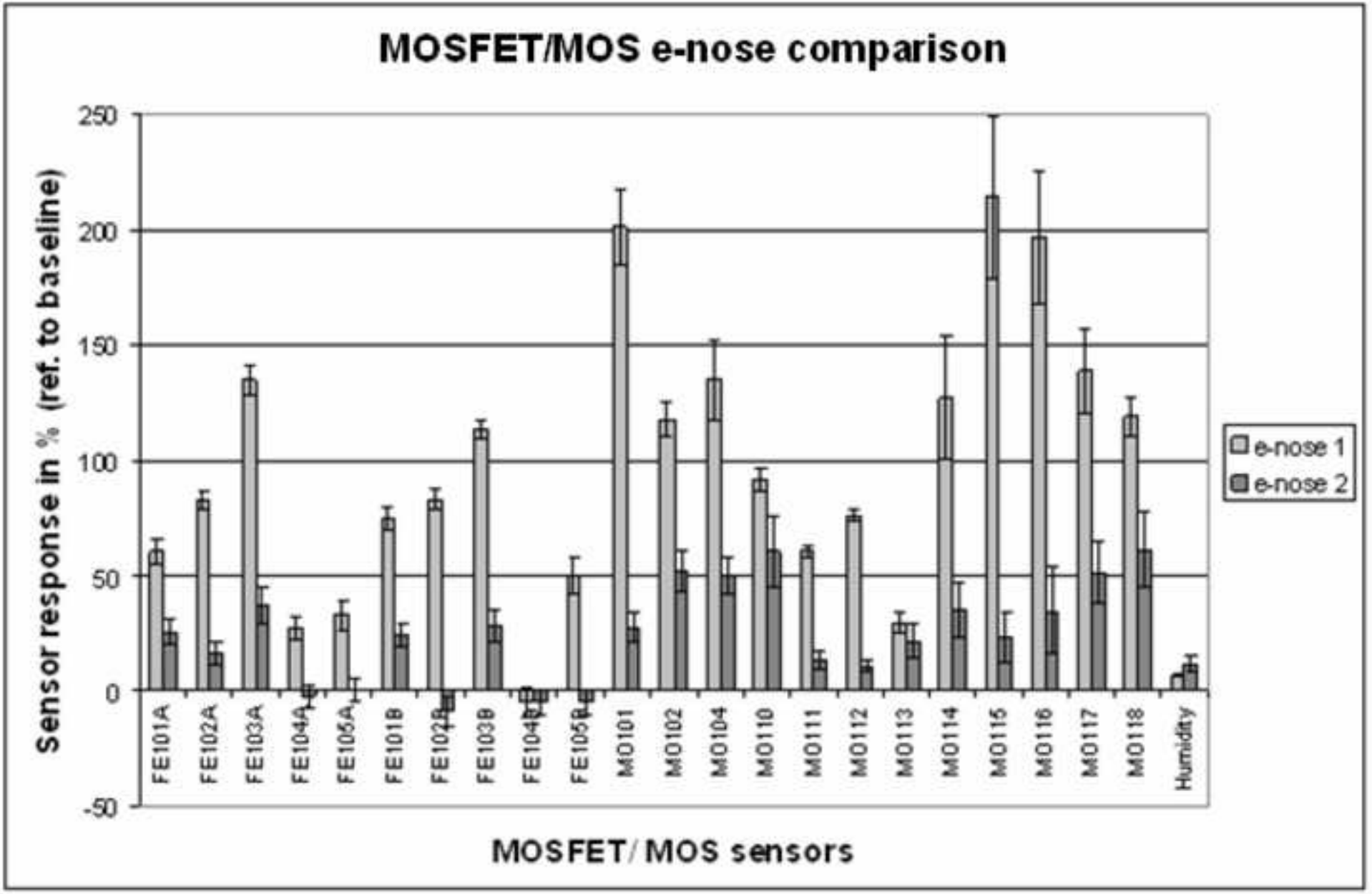

ROW and 2-butanol analysis using no filter, 0.45µm filter and a 0.20 µm filter
Scores on PC 2 (0.23%)
Scores on PC 1 (99.67%)
1 = no filter; ROW
2 = no filter; 2-butanol
3 = 0.45 µm filtered ROW
4 = 0.45 µm filtered 2-butanol
5 = 0.20 µm filtered 2-butanol
1
2
3
4
5

Figure(s)


Discrimination between 2 CP e-noses over time
PCA 2 (15.0686%)
PCA 1 (84.0348%)
CP e-nose 2
CP e-nose 1
4
3
2
1
0
-1
-2
-3
-4
-4
-3
-2
-1
0
1
2
3
4


Responses of CP e-nose 1 over time (tp)
Changes in divergence (%)
-50
-45
-40
-35
-30
-25
-20
-15
-10
-5
0
1
2
3
4
5
6
7
8
9
10
11
12
13
Sensors
tp 1
tp 2
tp 3
tp 4

Figure(s)


MOSFET/MOS e-nose comparison
Sensor response in % (ref. to baseline)
250
200
150
100
50
0
-50
FE101A
FE102A
FE103A
FE104A
FE105A
FE101B
FE102B
FE103B
FE104B
FE105B
MO101
MO102
MO104
MO110
MO111
MO112
MO113
MO114
MO115
MO116
MO117
MO118
Humidity
e-nose 1
e-nose 2
MOSFET/ MOS sensors

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METHODOLOGICAL VARIATION IN HEADSPACE ANALYSIS OF LIQUID SAMPLES USING ELECTRONIC NOSE

Henri Knobloch[1§], Claire Turner[1], Andrew Spooner[1], Mark Chambers[2]
[1]Cranfield Health, Cranfield University Cranfield, Bedfordshire, MK43 0AL, UK
[2] TB Research Group,Department of Statutory and Exotic Bacterial Diseases Veterinary Laboratories Agency Weybridge Woodham Lane New Haw, Addlestone Surrey KT15 3NB UK

§Corresponding author

Abstract

In past years, numerous electronic nose (e-nose) developments have been published describing analyses of solid-, liquid- or gaseous media in microbiological-, environmental-, agricultural- or medical applications. However, little has been reported about complex methodological pitfalls that might be associated with commercially available e-nose technology. In this paper, some of these pitfalls such as *temperature*, the *use of filters* and *mass flow* using different sampling methods (static- and dynamic sampling) are described for two generations of conducting polymer e-noses (ST114/214, CPs, both Scensive Tech. Ltd). A comparison with metal oxide semi-conducting field effect transistor/ metal oxide semiconductor (MOSFET/MOS) e-noses regarding *stability across replicates* and *over time* was made. Changes in temperature were found to give larger sensor responses, whereas the application of filters led to quantitative and qualitative changes in sensor responses due to a change in mass flow which was also affected by the sampling method. Static sampling provided more stable flows across replicates. Variation was investigated for CPs and MOSFET/MOS e-noses that gave different responses over time and across replicates. These methodological factors cause a lack of stability and reproducibility, demonstrating the pitfalls of e-nose technology and therefore limit their utility for discriminating between samples.

Keywords: Electronic nose, temperature effect, headspace, filter, sampling method

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1. Introduction

For more than 20 years, so-called electronic noses (e-noses) have been widely applied for headspace- and trace gas analysis from solid-, liquid- and gaseous samples. During this time, different sensing methods have been developed but the e-nose principle remained the same [1-5]. Generally, e-noses consist of an odour delivery system, an array of sensors, a data acquisition- and a data analysis unit. In all types of e-nose, the sensor array exploits the conversion of changes in electrical-, thermal-, mass- or optical properties into an analysable signal [6]. As for human noses, sensors show an overlapping specificity and therefore provide a non-quantitative sensor response, a so-called fingerprint, rather than a specific qualitative or quantitative answer. The lack of specificity is due to the fact that sensors react with functional groups and structures of analytes rather than specifically with the molecule itself [7, 8]. Sensor responses are then analysed using multivariate data analysis techniques in order to classify or discriminate a group of samples. But data analysis methods are complicated by the fact that there are multiple causes of variation –biological differences between samples or differences arising from the analysis itself (methodological variation). Methodological variation, including sensor drifts, may lead to unclear or contradictory results because the lack of repeatability or “disturbance” can be expressed as variation which may obscure biological variation. Hence the elimination of methodological variation is one of the biggest challenges associated with e-nose technology [9]. In this study, methodological influences associated with headspace analysis from liquids were investigated using two typical but different types of electronic nose systems; two portable conducting polymer sensor based e-noses (CPs) and two non-portable MOSFET/ MOS based e-noses. For each type, devices were compared.

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Temperature is the main influencing factor in generating and analysing headspace from liquids. As predicted by Henry's law, temperature affects the vaporisation of molecules. Even minor increases in temperature lead to a higher concentration of molecules in the gas phase. For the same reason, it also affects relative humidity. Variation of e-nose responses due to these parameters has been reported by various authors [10-13]. CP e-noses are particularly sensitive to humidity [14]. The electrical conductivity for CP e-noses is increased especially for electrophobic and nucleophilic substances [15]. Both monomer structure and inter-chain behaviour change [16] due to water vapour adsorption on the sensor surface. Filters have been applied to prevent sensor surface damage due to moisture and, in case of infectious material, to prevent contamination [17, 18].

Memory effects were also reported for CPs, in particular with gaseous compounds which have a strong proton affinity. The interaction with monomers leads to a further sensitivity loss [19]. Unlike MOSFET/ MOS e-noses, conducting polymer-based e-noses were also reported to be almost resistant to poisoning and to have a higher selectivity in comparison to semiconductors [10, 11]. Furthermore, the growth process for CP e-nose polymers is variable leading to different sensor arrays and responses [11, 20].

Regarding sampling methods, two different types can be distinguished: *static* and *dynamic*. The main difference between both methods is the constant pressure in the sampling apparatus for static sampling while pressure varies using dynamic sampling. Changes in pressure change the flow rate of molecules and therefore dynamic sampling may give more inconsistent results. Since dynamic sampling requires aerating the sample in order to compensate the pressure difference (e.g. lab air), sample dilution occurs leading to less sensitive analyses and temporal variability due to a non-equilibrium state (variation over replicates and runs) [12, 13, 21].

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For MOSFET and MOS sensor-based e-noses, the effect of the temperature of the sensors and temperature of the samples analysed strongly influence the responses [22]. However, since sensors were strongly temperature and humidity controlled these parameters were not included in this study. MOSFET and MOS sensors were only considered in terms of repeatability over replicates and time.

The purpose of this study was to examine methodological variation during sampling process which may influence sensor responses and therefore sample classification and discrimination. For two CP e-noses, the variability in sensor response due to changes in temperature, flow rates and experimental setups (usage of filters, different sampling techniques) was assessed. Furthermore, the repeatability of analyses (replicates, over time) was investigated for CP and MOS/MOSFET e-noses. E-noses of the same type were compared and variation during the sampling process was assessed using both types of e-nose.

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2. Materials and Methods

2.1 Apparatus

Two types of e-nose were used: conducting polymer- (CP) and metal oxide semi-conducting field effect transistor/ metal oxide semiconductor (MOSFET/MOS) e-noses.

The Bloodhound ST114 and the ST214 CP e-noses (both Scensive Tech. Ltd.) contained 14 conducting polymer sensors. The headspace is conveyed from the sampling container via a sampling port to the sensor array using an internal pump. The sensor array is not in a temperature and flow controlled environment. The time for adsorption and desorption during analyses was 20 seconds and seven replicates were analysed unless otherwise stated. The total time per replicate was 55 seconds.

Two MOSFET/ MOS e-noses (both NST 3220 Lab Emission Analyser; Applied Sensors, Linköping, Sweden) were assessed. The NST lab emission analyser houses ten MOSFET sensors (FE101A- FE105A, and FE101B- FE105B), twelve MOS sensors (MO101, MO102, MO104, MO110- MO118) and a humidity sensor. The principle of sampling is different from that of the ST214 e-noses. Samples are analysed from 30mL vials sealed with a septum and a lid which are placed in a rotating carousel and which maintains a constant temperature (e.g. 25°C) for sample equilibration. A double needle penetrates the septum and pumps headspace to the sensor panel where reversible adsorption processes take place at 140°C (MOSFET) and 170°C (MOS). The second needle maintains atmospheric pressure in the vial.

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2.2 Sampling methods

The effect of two types of sampling on the sensor response was investigated.

Dynamic sampling: Using this method, a liquid sample (5 mL) was pipetted into a 30 mL vial and directly connected to the e-nose sampling port. When the e-nose started sniffing the headspace, a pressure difference (Δp) was generated which was compensated for by an incoming air stream (e.g. lab air).

Static sampling: An inflatable Nalophan bag (KALLE, UK) was attached via a polypropylene tube with a Swagelok fitting (Swagelok, England) to a Luer counterpart. The Luer fitting could be directly connected to the e-nose. An internal pump delivered the headspace to the sensor array and the bag gradually collapsed due to the volume reduction. Nalophan is available in sleeves of different diameters and its length may be varied according to sample volume required. The material does not emit volatiles detectable by e-nose (data not shown). To compare different sampling methods, the ratio of the liquid sample volume and headspace air volume was calculated (V_{SAMPLE}/ $V_{HEADSPACE}$).

2.3 Assessing the effect of temperature on e-nose response

The Bloodhound ST214 e-nose used in this study lacks a temperature controlled environment. In order to demonstrate the effect of temperature on the e-nose, reverse osmosis water (ROW) and bovine serum were analysed at different temperatures. 0.5 mL of serum and ROW were pipetted into small Nalophan bags with a volume of 0.7 L to give the ratio of sample- to bag volume of 7E-04. The serum was obtained from one clinically healthy bovine animal (breed “Holstein”) held at the FLI (Friedrich Loeffler Institute, Jena, Germany). Both samples were incubated for 30 minutes and

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analysed at room temperature (21°C) and in a water bath (Type JB1, Grant Instruments, Ltd.) at 30°C and 40°C, respectively; ambient air was also analysed as a standard.

2.4 Mass flow and filters

Mass flows using different setups

The Bloodhound ST214 and ST114 e-noses used in this study lack a flow controller for adjusting mass flow. Hence, flow stability and rates were monitored under different sampling configurations using a mass flow sensor (Honeywell, AMW3300V). First, a Luer fitting was attached to the e-nose sampling port and to the mass flow sensor followed by a 0.45 µm pore sized Sartorious Minisart filter which was attached to the bag or sampling container. The mass flow rates for all combinations and both CPs were measured. The bag volume for static sampling was 0.7 L and the container volume for dynamic sampling was 30 mL. 1 mL of ROW or 5 mL of ROW were pipetted into the bag and the container as a standard solution, respectively. Flow rates were measured during the adsorption phase, performed in duplicate and given as averaged values. Temperature was kept constant at 25°C.

Mass flow stability for static sampling

The mass flow over eight successive replicates was monitored using static sampling. The experimental setup (calibration and analysis using two ST214) was similar to that described above with an increase in bag volume to 0.8L while the volume of a 10ppm 2-butanol standard solution was kept constant at 1mL (ratio: 1.25E-3). Time for adsorption was increased to 30 seconds.

Mass flows using different types of filter

The impact of two different filters, a 0.45 µm pore sized Sartorious Minisart filter and a 0.20 µm Whatman Acrodisc LC13 filter on the analysis of a 2% (v/v) 2-butanol solution was investigated. The changes to signal intensity (quantitative changes) and signal

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pattern (qualitative changes) of a CP e-nose (ST214) were studied. For comparison, ROW was also analysed either with no filter or the 0.45 µm filter. Eight replicates per experimental setup were taken. The temperature was kept constant at 25°C.

Selected ion flow tube mass spectrometry (SIFT-MS) was also used to investigate quantitative and qualitative changes in samples after passage through a filter. SIFT-MS is a real-time mass spectrometry method that provides quantitative data on compounds present. Full details are given in [23]. Duplicate sample bags containing 0.5 mL of bovine serum and filled with 0.7 L hydrocarbon-free air were analysed with and without a 0.20µm PVDF filter (Whatman, Acrodisc LC13). The concentrations of key marker compounds such as water, acetone, methanol and ammonia were measured.

2.5 E-nose comparison and characterisation

Conducting polymer e-nose characterisation

Variability of the two CPs from the same production batch (both ST214 e-noses manufactured under the same conditions within the same period of time) across four time points over a day was assessed using bovine serum sampled statically. Small bags (0.8 L) were used for each time point and device. The serum sample volume was 0.9 mL which leads to a corresponding sample to headspace volume ratio of 1.2E-3.
The temperature was maintained at 25°C. After incubating each bag for 15 mins at 25°C to establish an equilibrium, the bags were subsequently analysed (7 replicates) every two hours on four occasions beginning at 10:00 to assess the following factors:

- Device (describing variance caused by the e-noses)
- Time point (describing variance caused across time)
- Size of bag (describing differences according to bag size)

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- Replicates (describing differences due to repetitive sampling across one analysis)

MOSFET/ MOS e-nose characterisation

In order to characterise the MOSFET/MOS e-noses, the variation caused by different sample volumes and position around the carousel was investigated. Twelve vials containing six different sample volumes (50 µL, 100 µL, 150 µL 200 µL, 250 µL and 500 µL) of bovine serum were sampled twice to test reproducibility. After 15 minutes equilibration samples were analysed randomly with an air flow of 60 mL per minute. The adsorption time was 30 seconds.

The two MOS/MOSFET e-noses were also used to investigate differences between the devices and across time to compare characteristics with the CP e-noses. 200 µL of bovine serum was pipetted into six vials per time point (sample volume to vial volume ratio: 6.7E-3). Three vials were analysed randomly at four different time points starting at 10:30 in 2h intervals. The adsorption phase time was 30 seconds. Differences caused by devices, over time, and replicates were investigated.

2.6 Statistical analysis

In order to determine the influence of a parameter on the sensor response (divergence, maximum amplitude of signal in sample analysis) univariate data analysis techniques were used. Linear regression and multifactor ANOVA were performed using the statistical package SPSS (version 11.5, 2002). The level of significance for these analyses was $P \leq 0.05$ and the impact of each parameter on the result was given as t-value. For e-nose comparison, Principle Component Analysis (PCA) was performed using Matlab on the raw datasets and on auto-scaled as well as mean-centred data.

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Auto-scaling scales all values of a column of the data matrix by its standard deviation which eliminates different ranges or magnitudes of data. *Mean-centring* eliminates constant offsets by subtracting the column mean from the single values.

3. Results

3.1 Influence of temperature

Multifactor ANOVA and linear regression were performed on data obtained from the ST214 e-nose. The influence of temperature on signal traces of ROW and bovine serum was investigated.

Sensors 6 and 8 revealed significant differences between ROW and bovine serum (Figure 1). Serum samples led to more positive values in comparison to ROW; similar tendencies were observed for the other sensors but were not statistically significant.

These sensors were also influenced by *temperature*, hence a combination of both factors affected the sensor signals. Sensors 10 and 11 were neither influenced by the *substance* nor by *temperature*. The remaining sensors were significantly influenced by *temperature*. An increase in temperature led to more negative values (negative t-factor) so to a more negative change in divergence. Figure 1 illustrates the increase in signal intensity with increasing temperature. Ambient air data are also shown.

[INSERT figure 1 about here]

3.2 Mass flow and filters

Mass flows using different setups

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The mass flow rates under different experimental conditions were 180 and 200mL/min respectively when the Luer fitting, flow sensor and the bag fitting were attached to both e-noses (ST114, ST214). The flow rates for static sampling were 98% (ST114) and 97% (ST214) of the original mass flows rates, while for dynamic sampling, rates decreased to 66% for both devices. For dynamic sampling, during the adsorption phase, mass flow varied due to the bubbling effect. The addition of the 0.45µm filter led to a significant drop in flow to 65% and 68% for ST114 and ST214 using static sampling and to 52% and 57% for dynamic sampling.

Mass flow stability

Changes in mass flow were observed testing different *sampling methods* and *devices* for both CP e-noses when analysing eight replicates of a 10ppm butanol solution (ANOVA, $P \leq 0.05$). Constant flow rates were found for replicates 2 to 5 for e-nose 1 (set as 100%) and e-nose 2 but the second e-nose had a 17% higher flow rate. For replicates 1, a minor decrease in flow rates of 4% was found for both e-noses and approximately 72% of the headspace volume was analysed (573 mL e-nose 1 and 576 mL for e-nose 2). Flow rates decreased significantly by 15 and 13%, respectively.

The decrease in mass flow occurred with progressive collapse of the bag.

Mass flows using different types of filter

Comparing divergences obtained analysing the 2-butanol solution with no filter, a 0.45µm (Sartorious) and a 0.20µm filter (Whatman), significant differences were found for all sensors (ANOVA, $P \leq 0.001$, raw data not shown). The highest sensor responses, expressed as negative divergences, were seen when there was no filter (group 1, replicates 1 to 8). Attaching the 0.45 µm filter, the values became less negative (group 2, replicates 9 to 16); use of the 0.20 µm filter (group 3, replicates 17 to 24) led to lowest changes (see figure 2).

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[INSERT figure 2 about here]

Qualitative changes in signal pattern with the use of filters were investigated by comparing the results of the 2-butanol solution with those obtained analysing ROW (figure 3). Using no filter, both solutions could be distinguished while no discrimination was found using the 0.45 µm filter. Furthermore, the biggest discrimination was found using the 0.20 µm filter and the unfiltered butanol solution. Principle Component 1, covering 99.67% of all variance, mainly represented changes due to filter application rather than differences between the solutions.

[INSERT figure 3 about here]

Quantitative data from SIFT-MS analyses of duplicate cattle serum headspace samples showed changes in some key marker compounds. The concentration of water measured changed from a mean of 5.15 % for unfiltered samples to a mean of 4.26% for filtered (0.20 µm Whatman filter) samples (reduction of 17%); ammonia from a mean of 771 parts-per-billion (ppb) for unfiltered and 547 ppb for filtered (reduction of 29%); methanol from a mean of 424 ppb for unfiltered and 962 ppb filtered (increase of 126%). Acetone remained unchanged, at a mean of 656 ppb unfiltered and 680 ppb unfiltered. Clearly, the addition of a filter changed the concentration of many volatile compounds.

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3.3 E-nose comparison and characterisation

Conducting polymer e-nose characterisation

Sensor responses from all four time points obtained from similar CPs of the same type and manufacturer were analysed in Matlab using PCA. Principal Components 1 and 2 covered 99.10% of all variance. A clear discrimination between both e-noses was found using auto-scaled data. Without pre-treatment, data could not be interpreted.
The difference between the devices covered 84.03% of all variance, and 15.07% of all changes were due to changed divergences over time using static sampling (see figure 4).

[INSERT figure 4 about here]

Considering PCA plots, time points 1 and 3 as well as 2 and 4 were grouped together for CP e-nose 1. Similar results were found for e-nose 2 however, responses of time point 4 were more scattered.
SPSS analysis using linear regression and multifactor ANOVA ($P \leq 0.05$) showed that the *device*, the *time points* and the *replicates* were potential factors influencing the result. The factors *device* and *time point* had the biggest influence while factor *replicates* was almost not statistically relevant, except for two sensors.
Considering the responses over time for each device separately, divergences changed throughout the day for both e-noses (shown for one e-nose in figure 5). Sensor responses were highest at time point 1, decreased at time point 2 increased and decreased again for time points 3 and 4. Responses obtained at time points 1 and 3 as well as 2 and 4 were closest, respectively. Results for the other e-nose showed a similar trend. This result confirmed PCA findings.

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[INSERT figure 5 about here]

For almost all sensors and both devices the temporal variation appeared sinusoidal, and there was a tendency for responses to decline across all time points and for both e-noses; demonstrated using SPSS multifactor ANOVA ($P \leq 0.05$)..

MOSFET/ MOS e-nose characterisation

It was found that the factor *replicate* had a statistically significant influence on some sensor responses only ($P \leq 0.05$). A few MOSFET sensors (FE103A, 104A, 105A, 104B) showed a significant difference between the first and the second reading of the same vial. Most of the sensors showed a similar tendency of a decrease from *replicate 1* to *replicate 2* although these results were not significant.

The *sample volume* had no influence on the results within the volume range evaluated (50 to 500µL).

Analysing the averaged values across all *time points* and *replicates* for the two MOSFET/ MOS e-noses, there was a significant difference between the two. Except for the sensor FE104B, which was a MOSFET sensor, all other sensors demonstrated significantly different responses between the two devices. The first e-nose showed higher responses for MOSFET and MOS sensors (see figure 6).

[INSERT figure 6 about here]

The standard deviation from the base line for the first e-nose varied between 0.43 for the sensor MO111 (3.8% change in sensor response) and 35.22 for MO115 (16.4% change in sensor response). For the second e-nose, standard deviation was between 2.3 for the sensor MO112 and 19.0 for MO116 (54.0% change in sensor response). However,

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sensors with small responses varied up to 201%. The averaged standard deviation from the baseline was 9.9 for the first e-nose and 7.8 for the second device.

With the first e-nose, most of the MOSFET-generated values showed significant variation between the *replicates* using different vials, while samples analysed at different *time points* led to almost no changes. In contrast, the MOS sensors showed almost no changes between the *replicates* but data analysis indicated a significant decline in sensor responses over *time* for most sensors (multifactor ANOVA, $P \leq 0.05$). Only two sensor responses increased across time (MO111, MO112).

Analysing the same factors for the second e-nose, it was found that while the responses for all sensors were not affected by any variation in *replicates*, the traces of almost all MOS sensors were influenced by *time*. Generally, the responses decreased over the course of the day. In three cases the responses remained constant (MO101, MO102, MO104) and in another two cases the values increased across time (MO115, MO116). The MOSFET sensors generally exhibited a decrease of signal intensity across time but this tendency was only statistically significant in two cases (FE105A, FE103B).

4. Discussion.

4.1 The effect of temperature

Temperature changes had a major effect on sensor response. Even the two sensors able to discriminate between ROW and serum samples were significantly influenced by temperature. Changing temperature led to both qualitative and quantitative changes since a higher temperature leads to a greater concentration of volatiles in the headspace, which will generate different sensor responses. Such large changes in response due to

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temperature may mask the differences between samples, preventing discrimination or classification using multivariate techniques.

Changes in the physico-chemical properties of conducting polymers or their monomers when exposed to different temperatures have been described in literature. Impedance was decreased with an increase in humidity and temperature [10-13]. The temperature optimum was found to be 25°C [7, 24].

A temperature controller would add weight and bulk to the device. Some work has been carried out to construct a flow cell for temperature- and flow controlled analysis. Attempts were made to optimise the positions of the sensors on a heating block and the incoming gas so that headspace reacted in the same way with optimised sensors [25, 26]. With these changes in design, the most significant e-nose problems might be resolved to produce improved signal responses in terms of stability, reproducibility, response time and amplitude, all without losing e-nose portability.

4.2. Mass flow and filters and stability

Results of this study indicate that the previous assumption of an estimated mass flow rate of 200 ml/min using similar CP noses is not valid since flow rates change depending on sampling methods and filter usage. The ST114 had a flow rate of 180 mL/min and the ST214 of 200 and 235mL/min, respectively. A drop of 75% compared to the stable flow rates over replicates 2 to 5 was observed using dynamic sampling and the additional application of a 0.45 µm pore sized filter which was previously used in other studies [27, 28]. Bubbling air through the liquid sample led to variation and a decrease in pressure in the vial which was also found by others [29]. Dilution of the headspace and a non-equilibrium state led to variation between replicates also reported by others [30], while some authors obtained the best discrimination with

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dynamic sampling but better sensitivity using static sampling [6]. The filter was an additional resistance in flow rate for CP e-noses. In contrast, static sampling provided stable mass flow rates and equilibrium states because of the collapse of a sealed bag. Therefore, static sampling can significantly improve e-nose responses by minimising variation between and over replicates. In trace gas analysis (lower ppm to ppb), stability is crucial as even small changes may lead to non-discrimination.

Qualitative and quantitative changes in signals were found when filters were used. The change was dependent on the pore size of the filters, which protect the conducting polymer sensing surface of the e-nose from being changed by a high extent of water vapour. Mass flow rates decreased with a decline in pore size. However removal of water vapour will also remove other volatile compounds and leads to a change in headspace composition. This was confirmed using CP e-noses combined with PCA data analysis (clustering according to filter application) and analysing quantitative SIFT-MS data. Since most biological samples contain water (e.g. blood/serum, urine), filters have been widely used for protection of the sensors (0.45 µm PTFE, Whatman/Hepavent) [26-29]. However, these results indicate that using a filter may not a valid approach since discrimination between 2-butantol and ROW was not possible once a 0.45 µm filter was introduced and 98% of all variation was due to different filter application. Headspace was significantly changed.

Results of SIFT-MS analysis showed qualitative and quantitative changes in sample composition with the addition of a filter. The claimed advantage of using a filter for sensor protection [21, 30] is highly questionable since sensor response patterns change with the use of a filter showing changes in methanol or ammonia and therefore discrimination is made difficult.

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4.3 E-nose characterisation and comparison

Conducting polymer e-nose characterisation

Temporal changes in sensor response have been reported elsewhere [12, 13]. The sine wave-like changes described here across the day are possibly due to semi-reversible changes on the sensor surface. Since this effect was observed for both CP e-noses using static bag sampling and confirmed using multifactor ANOVA and PCA it seems to be a systematic problem which is not associated with dilution of samples nor a non-equilibrium state. It appears to be the result of semi-reversible desorption. Certain molecules might be desorbed before the software determines a new baseline (offset) for acquisition, meaning that in later analyses, responses cannot develop the same way since some binding sites are still occupied. So the changes in comparison to the baseline are different from the preceding analysis. Occupation of binding sites may come from water molecules of ambient air interacting with dopants or where headspace molecules were not desorbed properly. Purging with an inert gas such as nitrogen has been used by some authors to improve desorption and repeatability [7, 31]. The reasons why responses in this study were in the form of a sine wave, however, remain unclear. This may be evidence of memory effects which have been described by several authors [10, 19]. Further analyses are necessary to elucidate the cause of this effect. A comparison of sensor responses of similar CP e-noses produced by the same manufacturer was not possible due to different flow rates (180 mL/min to 235 ml/min) and qualitatively different responses, although the same substances were analysed under identical conditions (see section 2.5 and 3.3 CP e-nose comparison).

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MOSFET/ MOS e-nose characterisation

The sensor response patterns obtained from two MOSFET/ MOS e-noses were different when sampling the same headspaces. However, the variation was random and not significant; high standard deviation was found across various sample volumes (50 to 500 µL) but not across vials in the carousel. This was also confirmed in the literature [32, 33]. Almost all MOSFET sensors of e-nose 1 were found to be replicate dependent and almost all MOS sensors varied over time. The fact that different values were obtained (independent to the sample volume) when vials were sampled twice contradicts the findings of others that dual needle sampling was a reliable sampling method [34]. The reason for this could be the simple fact that after sampling, the liquid had been "outgassed" and lost a proportion of its volatile compounds.

Similar to the CP e-noses, temporal changes in MOS sensors indicate an insufficient desorption process; the new baseline being determined with molecules still attached to sensor surface resulting in a different response.

Comparison between e-nose devices

Comparing e-noses of the same type under different experimental setups, a lack of comparability was a surprising and worrying observation in this study and points to important manufacturing and engineering inconsistencies. Lack of reproducibility of sensor responses was exacerbated by the sampling principle, which mainly affected the portable CP e-noses.

Despite the fact CP e-nose and the MOSFET/ MOS e-nose were not comparable due to different sensing principles, there were changing sensor responses for both types of e-noses over time which were due to semi-reversible adsorption. Standardisation and improvement of production methods towards reliable and comparable sensors is

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urgently required, particularly for conducting polymer devices but also for MOSFET/ MOS sensors.

5. Conclusions

- Static sampling using the conducting polymer e-nose is better than dynamic sampling because of stable mass flow which leads to better repeatability.
- Filters are not recommended as they quantitatively change the mass flow rate and qualitatively change the sensor pattern which may lead to non-discrimination.
- Temperature and mass flow rate controlled sensor chambers could enhance the repeatability of e-noses and therefore improve discrimination and classification using multivariate data analyses.
- Incomplete desorption from sensors affects both conducting polymers and MOS sensors. Longer purging with inert gases may solve this problem and lead to more consistent responses over time.
- Because of the sources of variation discussed in this paper, multivariate data analysis techniques are only suitable for data obtained with stable e-nose systems; otherwise methodological variation may mask biological differences between samples. To overcome this problem, some authors suggest an absolute calibration for e-noses [3, 35] or a compensation at the data analysis level [9] but we believe in improving methodology first, since the calibration or reference points also shift but not necessarily in a linear or predictable way (due to changes in temperature, humidity, sampling methodology and memory effects). Improvements in devices are advisable such as static sampling in a controlled ambient environment, the introduction of controlled sensor chambers, and

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automated purging. This should minimise methodological variation and enhance the classification and discrimination of samples based on the biological variation which is present in all types of sample [36, 37]. Such improved and optimised e-noses may then be capable of fulfilling the considerable promise of this technology.

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ACCEPTED MANUSCRIPT

References

[1] P. Mielle, Electronic noses: Towards the objective instrumental characterization of food aroma, Trends in Food Science and Technology 7 (1996) 432-438.

[2] J.W. Gardner, Hyun Woo Shin, E.L. Hines, An electronic nose system to diagnose illness, Sens. Actuators, B, Chem 70 (2000) 19-24.

[3] A.P.F. Turner, N. Magan, Electronic noses and disease diagnostics, Nature Reviews Microbiology 2 (2004) 161-163.

[4] S. Nimmermark, Use of electronic noses for detection of odour from animal production facilities: a review, Water Science and Technology 44 (2001) 33–41.

[5] I.A. Casalinuovo, D. Di Pierro, M. Coletta, P. Di Francesco, Application of Electronic Noses for Disease Diagnosis and Food Spoilage Detection, Sensors 6 (2006) 1428-1439.

[6] J. Lozano, J.P. Santos, J. Gutierrez, J. , M.C. Horrillo, Comparative study of sampling systems combined with gas sensors for wine discrimination , Sens. Actuators, B, Chem 126 (2007) 616-623.

[7] M. Chimenti, D. De Rossi, F. Di Francesco, C. Domenici, G. Pieri, G. Pioggia, O. Salvetti, A neural approach for improving the measurement capability of an electronic nose, Measurement Science and Technology 14 (2003), 815-821.

[8] C. DiNatale, A. Macagnano, E. Martinelli, R. Paolesse, G. D'Arcangelo, C. Roscioni, A. Finazzi-Agrò, A. D'Amico, Lung cancer identification by the analysis of breath by means of an array of non-selective gas sensors, Biosensors and Bioelectronics 18 (2003), 1209-1218.

[9] C. Di Natale, E. Martinelli, A. D'Amico, Counteraction of environmental disturbances of electronic nose data by independent component analysis , Sens. Actuators, B, Chem 82 (2002), 158-165.

[10] S. Ampuero, J.O. Bosset, The electronic nose applied to dairy products: a review , Sens. Actuators, B, Chem 94 (2003), 1-12.

[11] D.J. Strike, M.G.H. Meijerink, M. Koudelka-Hep, Electronic noses – A mini-review, Fresenius J Anal Chem 364 (1999) 499–505.

[12] A., Nake, B. Dubreuil, C. Raynaud, T. Talou, Outdoor in situ monitoring of volatile emissions from treatment plants with two portable technologies of electronic noses, Sens. Actuators, B, Chem 106 (2005) 36-39.

[13] K.R. Kashwan, M. Bhuyan, Robust Electronic-Nose System with Temperature and Humidity Drift Compensation for Tea and Spice Flavour Discrimination, IEEE Asian

Accepted Manuscript

ACCEPTED MANUSCRIPT

Conference on Sensors and International Conference on New Techniques and Biomeldical Research 2005

[14] D. Hodgins, The development of an electronic 'nose' for industrial and environmental applications, Sens. Actuators, B, Chem 27 (1995) 255-258.

[15] O.N. Timofeeva, B.Z. Lubentsov, Y. Z. Sudakova, D.N. Chernyshov, M.L. Klddekel, Conducting polymer interaction with gaseous substances: I Water, Synthetic Metals 40 (1991) 111-116.

[16] B.Z. Lubentsov, O.N. Timofeeva, S. Saratovskikh, V. Krinichnyi, A. Pelekh, V. Dmitrenko, M.L. Khidekel, The study of conducting polymer interaction with gaseous substances IV. The water content influence on polyaniline crystal structure and conductivity, Synthetic Metals 47 (1992) 187-192.

[17] R. Fend, C. Bessant, A.J. Williams, A.C.Woodman, Monitoring haemodialysis using electronic nose and chemometrics, Biosensors and Bioelectronics 19 (2004) 1581-1590.

[18] A.K. Pavlou, N. Magan, C. McNulty, J.M. Jones, D. Sharp, J. Brown, A.P.F. Turner, Use of an electronic nose system for diagnoses of urinary tract infections, Biosensors and Bioelectronics 17 (2002) 893-899.

[19] A.L. Kukla, Y.M. Shirshov, S.A. Piletsky, Ammonia sensors based on sensitive polyaniline films, Sens. Actuators, B, Chem 37 (1996) 135-140.

[20] A.C. Partridge, Y.P. Harris, M.K. Andrews, High sensitivity conducting polymer sensors, Analyst 121 (1996) 1349-1353.

[21] A.D. Wilson, D.G. Lester, C.S. Oberle, Development of Conductive Polymer Analysis for the Rapid Detection and Identification of Phytopathogenic Microbes, Techniques 94 (2004) 419-431.

[22] E.L. Kalman, A. Löfvendahl, F. Winquist, I. Lundström, Classification of complex gas mixtures from automotive leather using an electronic nose, Anal Chimica Acta 403 (2000) 31-38.

[23] D. Smith, D. P. Španěl, P. Selected ion flow tube mass spectrometry (SIFT-MS) for on-line trace gas analysis, Mass Spectrometry Reviews 24 (2005) 661-700.

[24] J.H. Cho, J.B. Yu, J.S. Kim, S.O. Sohn, D.D. Lee,J.S. Huha, Sensing behaviors of polypyrrole sensor under humidity condition, Sens. Actuators, B, Chem 108 (2005) 389-392.

Accepted Manuscript

ACCEPTED MANUSCRIPT

[25] M. Facitelli, A. Benassi, F. Di Francesco, C. Domenici, L. Marano, L., G. Pioggia, Fluid dynamic for simulation of a measurement chamber for electronic noses, Sens. Actuators, B, Chem 85 (2002) 166-174.

[26] F. Di Francesco, M. Falcitelli, L. Marano, G. Pioggia, A radially symmetric measurement chamber for electronic noses Sens. Actuators, B, Chem 105 (2005) 295-303.

[27] R. Fend, R. Geddes, S. Lesellier, H.-M. Vordermeier, L.A.L. Corner, E. Gormley, E. Costello, R.G. Hewinson, D.J. Marlin, A.C. Woodman, M.A. Chambers, Use of an Electronic Nose To Diagnose Mycobacterium bovis Infection in Badgers and cattle, J Clin Microbiology 43 (2005) 1745-1751.

[28] N. Magan, A.K. Pavlou, I. Chrysanthakisa, Milk-sense: a volatile sensing system recognises spoilage bacteria and yeasts in milk. Sens. Actuators, B, Chem 72 (2001) 28-34.

[29] S. Ampuero, S. Bogdanov, J.-O. Bosset, Classification of unifloral honeys with an MS-based electronic nose using different sampling modes: SHS, SPME and INDEX, Eur Food Res Technology 218 (2004) 198-207.

[30] T.H.H. Misselbrook, P.J. Hobbs. K.C. Persaud, Use of an Electronic Nose to Measure Odour Concentration Following Application of Cattle Slurry to Grassland, J Agr Eng Res 66 (1997) 213-220.

[31] W. Bourgeois, R.M. Stuetz, Use of a chemical sensor array for detecting pollutants in domestic wastewater, Water Research 36 (2002) 4505-4512.

[32] S. Benedetti, C. Pompei, S. Mannino, Comparison of an Electronic Nose with the Sensory Evaluation of Food Products by “Triangle Test”, Electroanalysis 16 (2004) 1801-1805.

[33] M. Morvan, T. Talou, J.-F. Beziaub, MOS–MOSFET gas sensors array measurements versus sensory and chemical characterisation of VOC’s emissions from car seat foams, Sens. Actuators, B, Chem 95 (2003) 212-223.

[34] M. Markelov, O.A. Bershevits, Methodologies of quantitative headspace analysis using vapor phase sweeping, Anal Chimica Acta 432 (2001) 213-227.

[35] T.D. Gibson, O. Prosser, J.N. Hulbert, R.W. Marshall, P. Corcoran, P. Lowery, E.A. Ruck-Keene, S. Herond, Detection and simultaneous identification of microorganisms from headspace samples using an electronic nose, Sens. Actuators, B, Chem 44 (1997) 413-422.

Accepted Manuscript

ACCEPTED MANUSCRIPT

[36] W.J. Harper, W. J., The strengths and weaknesses of the electronic nose Advances in Exp Medicine and Biology 488 (2001) 59-71.

[37] P. Van Geloven, M. Honore, J. Roggen, S. Leppavuori, T. Rantala, The influence of relative humidity on the response of tin oxide gas sensors to carbon monoxide, Sens. and Actuators, B, Chem 4 (1991) 185-188.

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Biographies

Henri Knobloch (MPhil), studied biotechnology at the University of Applied Sciences, Jena, (Germany) and did his first degree in non-invasive analysis, method development and standardisation of H_2O_2 concentrations in exhaled breath condensate and blood. He is currently a third year PhD student at Cranfield Health/ Cranfield University (UK). His research interests are trace gas analysis and the application and evaluation of electronic nose technology as a diagnostic tool.

Claire Turner (BSc. Hons., Ph.D, Dip. Comp., MIBiol) completed a PhD in the Department of Chemical and Biochemical Engineering at University College, London in 1993, and since then, has had an interest in the monitoring of biological systems. She is currently a Lecturer and Head of the Volatiles Research Group, Cranfield Health, Cranfield University and has a particular interest in the potential for VOCs from body fluids to be used to diagnose disease.

Spooner, Andrew (MPhil), completed his degree in Physics at Warwick University. He is currently a PhD student at Cranfield University (UK) and his research interests are in chemometrics and classification modelling for diagnosis of disease.

Mark Chambers, (BSc. Hons, PhD) obtained his Ph.D at Cambridge University and has held post-doctoral positions at Imperial College of Science, Technology & Medicine. In 1996 he joined the Veterinary Laboratories Agency, an Executive Agency of the UK Government Department for Environment, Food and Rural Affairs where he leads a team of eight scientists working on the development of vaccines, immunological reagents, and novel diagnostic assays for tuberculosis.

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Knobloch, Henri; Turner, Claire; Spooner, Andrew; Chambers, Mark

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