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IDENTIFICATION OF ODOR OF VOLATILE ORGANIC COMPOUNDS USING CLASSICAL SENSORY ANALYSIS AND ELECTRONIC NOSE TECHNIQUE

To methods of analysis of odor of volatile organic compounds have been compared: classical sensory analysis and electronic nose technique. Eight volunteers participated in two-week classical sensory tests. The instrumental odor analysis involved a prototype of 6-sensor electronic nose designed by the authors. This device provided higher reproducibility and reliability of the results as compared to the ones obtained via the sensory analysis. Three compounds of specific odor and differing in chemical structure were utilized in the studies: 1-propanol, benzaldehyde and 2,3-butanedione. Aqueous solutions of the compounds and their mixtures were prepared at three concentration levels: 50 ppb, 1 ppm, 50 ppm v/v. The electronic nose technique, unlike the classical sensory analysis, made it possible to differentiate between particular solutions below the level of odor perceptibility and also allowed differentiation between the solutions of similar odor intensity reported by the volunteers.

1. INTRODUCTION

Odor noxiousness in the vicinity of air pollution sources is the main reason of complaints from the local inhabitants. It is mainly connected with health hazard and discomfort caused by odor. Currently the only legal regulations concerning the problem of odor and the methods of its evaluation are contained in the *Environmental Law* of 27th April 2001 [1]. According to an international standard *an odor is an organoleptic property recognized using olfactory organ during inhalation of certain volatile substances* [2]. Odor is the information transferred in a chemical way, the importance of which is equal to the verbal communication. Sending information via odor is one of

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the oldest and the most important “inventions” of the nature [3]. A reliable research on the properties of odors is a very challenging task for analytical chemists [4]. Thus the question is: how to analyze odor? Is there an ideal method of odor analysis?

A source of valuable information on the properties of particular odor could be analysis of volatile compounds performed via the classical sensory analysis or with the use of instrumental techniques including chromatographic methods and electronic nose technique [5]. The classical sensory analysis is based on evaluation of odor by a trained group of people, performed in stable and controlled conditions. It is the oldest method of odor evaluation. Although the fundamentals are simple, this method is troublesome in implementation. Strict requirements enforce creation of the laboratories devoted exclusively to the sensory analysis, so the cost of a single analysis is high [6–8]. Even rigorous conformity with the evaluation procedures, high quality reagents, advanced laboratory equipment, efficient air-conditioning and ventilation do not guarantee satisfactory, unequivocal results. The result of such analysis depends on many factors including individual odor perceptibility of a particular person involved in the evaluation. Moreover, the sense of smell is very sensitive to external factors. One’s perception of odor can be different every day depending on mood, incidental situations occurring on the way to work, etc.

Implementation of the standard PN-EN 13725 *Air Quality-Determination of Odor Concentration by Dynamic Olfactometry* in the field of odor analysis unified the way the concentration of odor compounds is determined. Application of the dynamic olfactometry method allows objective evaluation of concentrations of gas mixtures in odor units/m³. Despite these advantages, it is still difficult to find a relation between the structure of chemical compounds and their odor. There are numerous odor similarities in nature, which in fact originate from the compounds differing significantly in chemical structure, for instance both nitrobenzene and hydrogen cyanide reveal bitter almond smell. Apart from aforementioned disadvantages of human sense of smell such as subjectivity, individual threshold of odor perception, emotional condition, there are other limitations such as susceptibility to adaptation and fatigue. The instrumental methods lack the above shortages, moreover, they can be superior in terms of improved selectivity, limit of detection or identification of the substances human senses are insensitive to. That is why their utilization for odor analysis is justified [9].

Two approaches dominate in the instrumental analysis of gaseous samples. The former one consists in identification of volatile aroma compounds using gas chromatography (GC), the other is based on “holistic” analysis of gaseous mixture without separation into its individual components. This approach engulfs electronic nose technique [10–14].

The electronic nose is a device comprised of a set of sensors selective towards given group of volatile compounds [15–19]. It is equipped with a reference-comparative system able to identify simple and complex odors. Artificial noses are cheap in service and fast in operation, so they constitute an alternative to relatively

expensive methods of gaseous samples evaluation: gas chromatography-mass spectrometry (GC-MS) or gas chromatography-flame ionization detector (GC-FID) techniques. Another advantage of these devices is high repeatability and reproducibility of results of analysis as well as possibility of miniaturization and thus production of portable equipment [20]. Disadvantages of chromatographic techniques include: high cost of analysis, time consumption, labour consumption and susceptibility to the method of sample preparation. The main shortages of the electronic nose technique are: metrological parameters of the applied sensors, complicated mathematical and statistical background implemented for measurement data analysis, necessity of database creation and training in order to interpret the results correctly.

The paper presents potentialities of the electronic nose as a tool for identification of particular organic aroma compounds and their mixtures differing in functional group and compares this approach with the classical sensory analysis. A prototype of electronic nose designed by the authors was able to identify and differentiate solutions of aroma compounds: 1-propanol, benzaldehyde and 2,3-butanedione, at the concentration level of 50 ppb, 1 ppm and 50 ppm (v/v).

2. IDENTIFICATION OF ODOR

2.1. IDENTIFICATION OF ODOR BY HUMAN SENSE OF SMELL

The sense of smell is one of the two chemical senses, the second one is the sense of taste. Its essence relies on an ability of identification of odor of particular chemical compounds or their mixtures present in the environment. An important element of the olfactory organ is the olfactory epithelium present on both sides of the nasal cavity. It consists of neurosensory cells, basilar cells and nurse cells. Human olfactory epithelium occupies the area of ca. 1–3 cm² and is located in the upper part of the nasal cavity. It is co-responsible for comfort, influencing significantly the nervous system. A substance generating the odor should be characterized by relatively high vapor pressure, relatively low polarity and should be partially soluble in water and in fats. It was also observed that substances inducing smell sensation were organic compounds of low molecular mass. The mechanism of acquisition of odor stimulus has not been fully recognized. Nevertheless, its four major stages can be distinguished (Fig. 1).

In the first stage, the olfactory receptors present in a cell membrane of the cilia fixed on the surface of the olfactory epithelium combine aroma molecules with nasal secretion. This phenomenon initiates transmission of the electrical signal in a cell thus activating cytoplasmic G proteins. The signal is then transmitted through the axons to the olfactory bulb present in brain, further to the olfactory cortex and higher cerebral



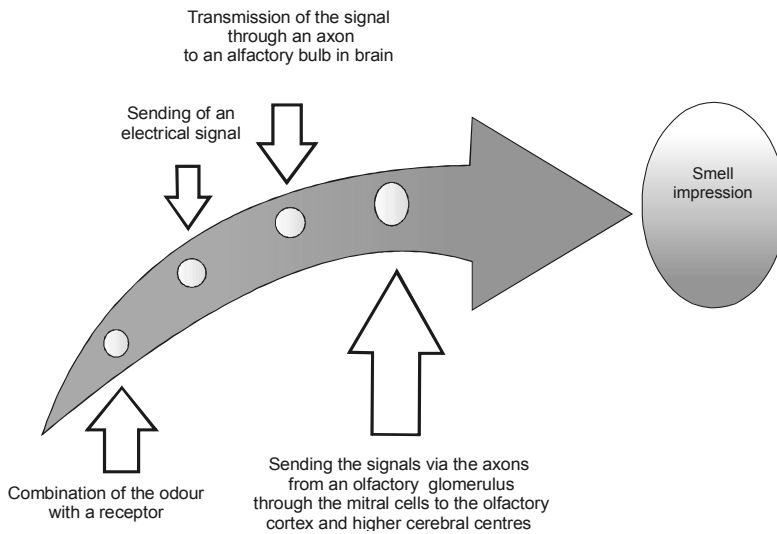


Fig. 1. Stages of odor stimulus acquisition by human sense of smell

centers, where perception of odor occurs – namely analysis of the properties of a sensation enabling identification of odor and evaluation of its intensity.

2.2. IDENTIFICATION OF ODOR WITH ELECTRONIC NOSE

Operation of the electronic nose resembles human sense of smell. The similarity is the fact that the sensors are the counterparts of the receptor proteins in nasal epithelium converting chemical information into analytically useful signal. The signal is sent to an identification system, which is brain in the case of human or appropriate mathematical-statistical algorithm as far as the electronic nose is concerned.

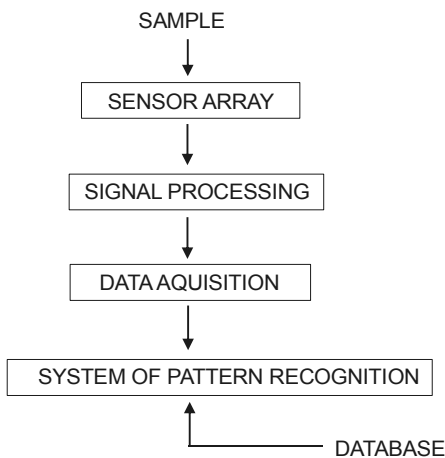


Fig. 2. Schematic description of a principle of the electronic nose operation



The electronic nose device (Fig. 2) consists of four fundamental and independent elements. The first is the sampling system, which eliminates all undesired factors which may interfere with sensor response and/or destabilize measurement environment. Stability depends on the parameters such as temperature and humidity being continuously controlled both in a sample compartment as well as in a sensors compartment. Moreover, the sample compartment must be made of inert and non-absorbent materials in order to prevent so-called wall memory effect. The second element is a detection system comprised with a set of chemical sensors. Generally, every sensor reveals different selectivity and sensitivity towards particular components of the sample, however as a whole they generate characteristic profile of chemical image of gaseous mixture. The third element of the electronic nose device is a data collection system, where the signal is converted and averaged. The last element is a system of identification of chemical images. It assigns every image to a particular class of references. The characterization of odors is based on a degree of similarity of the properties to a particular group of references. In the next step, a probability of correct odor identification is calculated. The simplest way of odor identification is graphical presentation of the results as the histograms of raw data called the aroma fingerprints. The most frequently applied methods of classification are principal component analysis (PCA) and artificial neural networks (ANN) [3, 13]. PCA is based on distribution analysis of variation of data originating from multiple measurements. Then new, orthogonal with respect to each other directions in space are found, which allow clear exemplification of these variations. Presentation of data in two- or three-dimensional plot makes it possible to visualize concentrations of the objects revealing similar properties. Typically first two components allow correct differentiation of the samples.

3. EXPERIMENTAL

Sample preparation. The solutions of aroma substances were prepared by the methods described elsewhere [21]. In order to describe odor intensity of selected substances by the comparative method, 10 aqueous solutions in of *n*-butanol the concentration range from ca. 6 ppm v/v to 80 000 ppm v/v (0.08 vol. %) were used as a reference. The reference solutions (numbered from 1 to 10) were prepared by the successive dilution method. Their concentrations are given in Table 1.

Table 1

Concentrations of *n*-butanol in reference solutions

Solution	1	2	3	4	5	6	7	8	9	10
Concentration in ppm v/v	80 000	28 000	9800	3430	1200	420	147	52	18	6



Three compounds of specific odor and differing in chemical structure were utilized in the studies: 1-propanol (A), benzaldehyde (B) and 2,3-butanedione (C). Their aqueous solutions were prepared at three concentrations: 50 ppm v/v (solutions A.1, B.1, C.1 and their mixture ABC.1 where concentration of each component was 50 ppm v/v), 1 ppm v/v (solutions A.2, B.2, C.2 and their mixture ABC.2 with concentrations of each component of 1 ppm v/v) and 50 ppb v/v (solutions A.3, B.3, C.3 and their mixture ABC.3 with concentrations of A, B and C of 50 ppb v/v). The chemicals (Sigma-Aldrich) were of p.f.a. grade. Deionized water from Mili-Q A10 device by Millipore was used as a solvent.

Procedure of sensory analysis. 20 volunteers participated in preliminary investigations which utilized aqueous solutions of *n*-butanol prepared at 5 concentrations: 0 ppm, 5 ppm, 10 ppm, 20 ppm, 40 ppm v/v. During 2 days, each volunteer carried out 10 analyses aimed at identification of an individual perceptibility threshold with respect to *n*-butanol solutions. The preliminary investigations allowed selection of volunteers, who fulfilled the criterion of individual repeatability required (1):

$$10^s \leq 2.3 \quad (1)$$

where *s* – standard deviation of individual odor evaluations.

8 volunteers (4 women and 4 men) aged 20–30 were selected to participate in the sensory analysis. They were trained for one week before the tests. The volunteers were non-smokers and their physical as well as mental condition was evaluated as very good. They have not been eating and drinking for an hour before the test in order to avoid interference from foreign odors with the aroma substances under examination. The tests were repeated 6 times during 2 weeks and after two month break the second series of tests was launched. The total number of evaluations was 576 in a single investigation series. The first task volunteers had to face was identification of an individual perceptibility threshold for the reference – *n*-butanol. Inhaling the reference solutions of *n*-butanol, starting from the solution No. 10 of the lowest concentration, the volunteer identified the solution, which he/she was able to smell as the first one. The next step was to compare the odor intensity of the reference with the solutions of substances A, B, C and their mixtures ABC. The aim was to find such solution of *n*-butanol, the odor intensity of which was close to the odor intensity of a given analyte.

Electronic nose technique. The experimental set-up consisted of a container with carrier gas (compressed air of N 5.0 purity, Linde Gaz Polska, Ltd.) connected via a Teflon tube 4 mm in diameter with a flow meter 2150 series by Tecfluid. The carrier gas flow rate was 5 dm³/h. The flow meter was connected with a “petit coat” bubbler, inside of which barbotage of liquid sample occurred. Volatile fraction formed was directed to the electronic nose prototype via a Teflon tube (Ø 4 mm). The prototype of the electronic nose was built from 6 semiconductor sensors by FIGARO company



(TGS 880, TGS 825, TGS 826, TGS 822, TGS 2610, TGS 2602). The bubbler, connecting tubes and the module with the sensors were in a thermostatic casing. The temperature was maintained at 20.0 ± 0.5 °C. Relative humidity of air inside the module was $82 \pm 1\%$. A conversion of the particular sensor output signals to digital signals was accomplished via a dedicated miniaturized integrated circuit. This set-up consisted of the sensor (operating in voltage divider arrangement), termination resistance selected for a particular sensor, adjustable amplification unit and zero system with adjustable voltage offset. The purpose of the measuring system was to convert changes of sensor resistance into voltage signal measurable by an analogue-digital converter. The outcome of this step was a voltage signal, whose changes corresponded in the entire measurement range of the converter to complete changes of the sensor resistance. The voltage obtained was converted into digital form in the range from 0 to 14 bit. The results were interpreted in terms of a function being the ratio of a particular sensor signal to the maximum signal (S/S_{\max}), which was digital information (voltage obtained from a particular sensor) divided by 14 bit.

Data was recorded 30 s after the measurement started (at that time concentration of the analyte in gas phase was maximum) and subject to preliminary processing using dedicated software. Six measurements were carried out for each sample (also after the two week period). The signal of each sensor (Figs. 3–9) was a mean from six measurements. Reproducibility of the results was at the level of 4.8–5.5% *CV* (coefficient of variation – the ratio of the standard deviation to mean value of signal of a particular sensor). Chemometric analysis of data was performed utilizing a commercially available software SAS Enterprise 4.3 with implemented PRINCOMP algorithm. The algorithm was employed for chemometric calculations using PCA. The structural elements of the electronic nose device, the modules for thermal stabilization of a sample during barbotage process and the modules for temperature and relative humidity stabilization of the air containing analyte are subject to a patent application.

4. RESULTS

4.1. CLASSICAL SENSORY ANALYSIS

The volunteers did not identify the odor of *n*-butanol solution No. 10 (the concentration of *n*-butanol of 6 ppm v/v was below their perceptibility threshold level). They pointed to the solution No. 9 as the one, at which the first smell sensations occurred (the individual smell perceptibility threshold level was defined). Odor intensity of the solutions A, B, C and their mixtures ABC was marked as 0 when a particular volunteer identified his/her individual smell perceptibility threshold level. Each solution of *n*-butanol with the number higher than the one, at which the individual smell perceptibility threshold level had been identified, was assigned the value higher by one. In the



case of the sensory analysis of the solutions A, B, C and their mixtures ABC the volunteers described odor intensity comparing it to the odor intensity of the *n*-butanol solutions marked from 9 to 5 (odor intensity scale was from 0 to 4). Table 2 presents information on odor intensity of the solutions of substances A, B, C and their mixtures ABC compared to the odor intensity scale of *n*-butanol solutions.

Table 2

Information on odor intensity of the solutions A, B, C and their mixtures ABC

Aqueous solution		Odour intensity
Symbol	Composition	
A1	50 ppm v/v 1-propanol	1.0
A2	1 ppm v/v 1-propanol	0.5
A3	50 ppb v/v 1-propanol	N
B1	50 ppm v/v benzaldehyde	2.0
B2	1 ppm v/v benzaldehyde	N
B3	50 ppb v/v benzaldehyde	N
C1	50 ppm v/v 2,3-butanedione	1.5
C2	1 ppm v/v 2,3-butanedione	1.0
C3	50 ppb v/v 2,3-butanedione	N
ABC1	50 ppm v/v 1-propanol, 50 ppm v/v benzaldehyde, 50 ppm v/v 2,3-butanedione	3.0
ABC2	1 ppm v/v 1-propanol, 1 ppm v/v benzaldehyde, 1 ppm v/v 2,3-butanedione	1.5
ABC3	50 ppb v/v 1-propanol, 50 ppb v/v benzaldehyde, 50 ppb v/v 2,3-butanedione	0

48 reports of smell sensations for each A, B, C solution and their mixtures ABC at a given concentration level had been arranged into an increasing series and a median of odor intensity was determined for that series. The N mark was assigned to the solutions, the odor intensity of which was below the individual smell perceptibility threshold level. Analyzing the results, it can be stated that 100% of volunteers identified the odor of the solutions of the substances A, B, C and their mixtures ABC at a concentration level of 50 ppm v/v regardless of the type of solution. Odor intensity of the solutions of lower concentrations depended on a type of compound. A decrease in odor intensity with a decrease in concentration was also observed. The highest odor intensity with respect to individual smell perceptibility threshold level revealed the mixture ABC.1 with concentrations of components equal to 50 ppm v/v. Benzaldehyde solution of concentration 1 ppm v/v was not detected by the volunteers, unlike the solutions of 1-propanol and 2,3-butanedione of the same concentration, the intensity of which was 0.5 and 1, respectively (compared to *n*-butanol solution). Samples A.3, B.2, B.3 and C.3 were not detected by the participants of the test. Whereas, the samples A.1 and C.2 were characterized by identical odor intensity corresponding to the level 1 of the reference scale. Intensity of odor of the samples C.1 and ABC.2 the was equal to 1.5.

4.2. PROTOTYPE OF THE ELECTRONIC NOSE

Figures 3a–d present 3D aroma fingerprints obtained from the analysis of A.3, B.2, B.3, C.3 and *n*-butanol No. 10 solutions using the electronic nose technique. Odor intensity of these solutions was below individual smell perceptibility threshold level of the participants of the classical sensory analysis. For all the aroma fingerprints one can observe lower values of sensors' signals as compared to the sensors' signals obtained during analysis of volatile fraction of solution No. 10. As opposed to the classical sensory analysis, application of the electronic nose technique enabled differentiation between the solutions A.3, B.2, B.3, C.3 and solution No. 10.

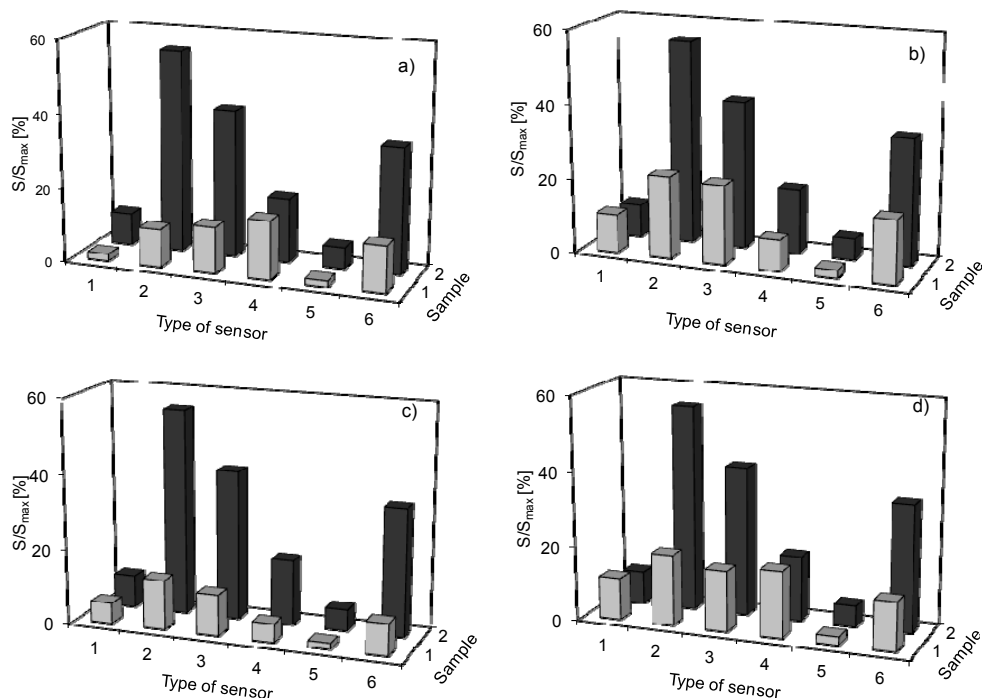


Fig. 3. Mean ratio of the signal of response electronic nose sensors to maximum signal versus sensor type and type of solution: a) A.3 (bright bars)–*n*-butanol No. 10 (dark bars), b) B.2 (bright bars)–*n*-butanol No. 10 (dark bars), c) B.3 (bright bars)–*n*-butanol No. 10 (dark bars), d) C.3 (bright bars)–*n*-butanol No. 10 (dark bars). Type of sensor: 1 – TGS 880, 2 – TGS 825, 3 – TGS 826, 4 – TGS 822, 5 – TGS 2610, 6 – TGS 2602

Moreover, comparing the results of PCA of volatile fraction of the solutions A.3, B.2, B.3, C.3 and the solution No. 10 (Fig. 4) one can observe differentiation between the odor samples. It is mainly due to the fact that sensors' response signal depends on the type of investigated substance (its chemical structure). On the other hand, similari-



ty of the aroma fingerprints B.2 and B.3 (Fig. 5a, b) stems from the fact that the analysis of volatile fractions was carried out for the same compounds but with different initial concentration.

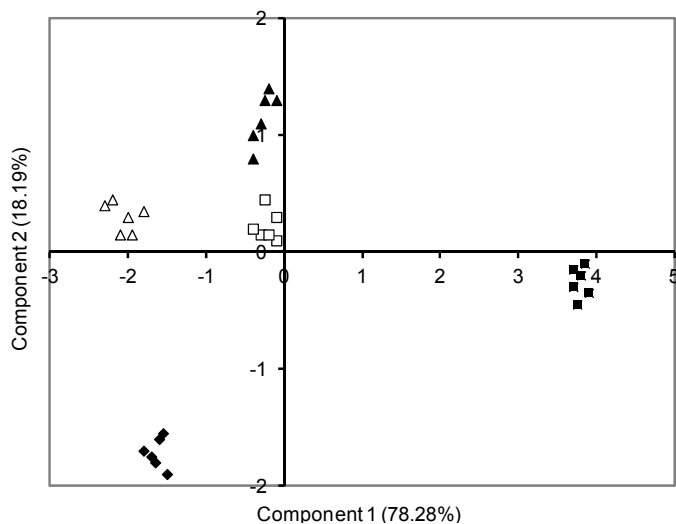


Fig. 4. PCA of volatile fractions of the substances: A.3 (◆), B.2 (▲), B.3 (△), C.3 (□) and *n*-butanol solution No. 10 (■)

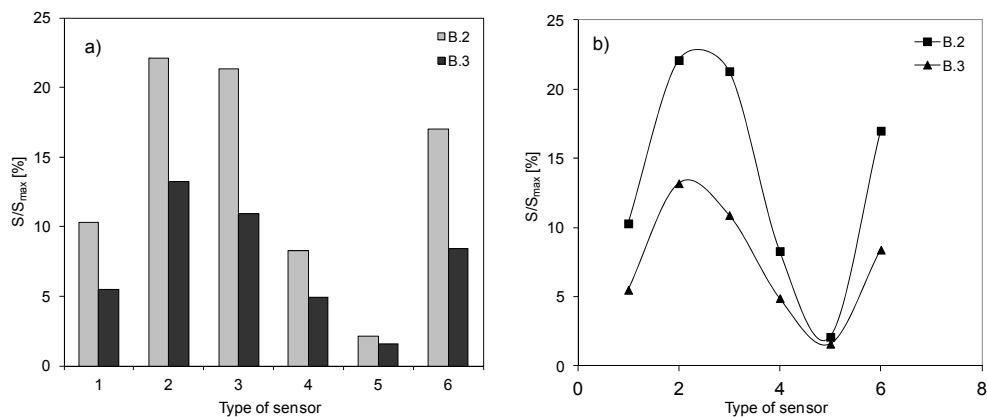


Fig. 5. Mean ratio of the response electronic nose sensors' signal to maximum signal versus sensor type and type of solution: B.2, B.3, a) bar plot, b) linear plot. Type of sensor: 1 – TGS 880, 2 – TGS 825, 3 – TGS 826, 4 – TGS 822, 5 – TGS 2610, 6 – TGS 2602

For the three solutions A.1, C.2 and solution No. 8, the odor intensity was identified as 1 (these solutions induced similar smell sensations precluding their differentia-

tion). Figures 6a, b depict 3D aroma fingerprints obtained from the analysis of A.1, C.2 solutions and solution No. 8. Also in this case the aroma fingerprints of the solutions of the substances A and C, obtained using the electronic nose technique, differed from the aroma profile of *n*-butanol solution.

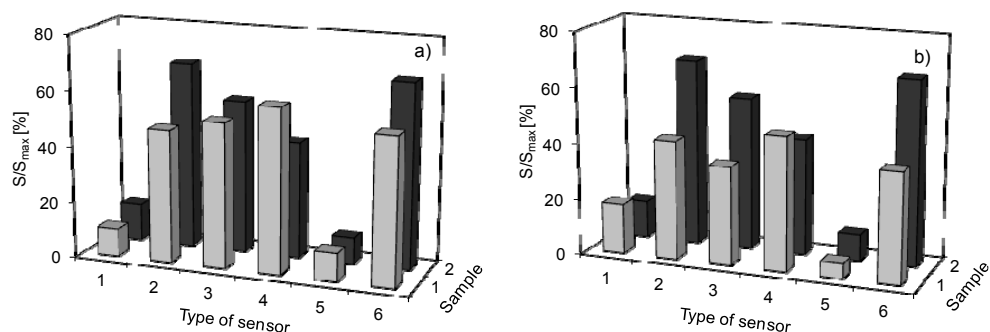


Fig. 6. Mean ratio of the response electronic nose sensors' signal to maximum signal versus sensor type and type of solution: a) A.1 (bright bars)–*n*-butanol No. 8 (dark bars), b) C.2 (bright bars)–*n*-butanol No. 8 (dark bars). Type of sensor: 1 – TGS 880, 2 – TGS 825, 3 – TGS 826, 4 – TGS 822, 5 – TGS 2610, 6 – TGS 2602

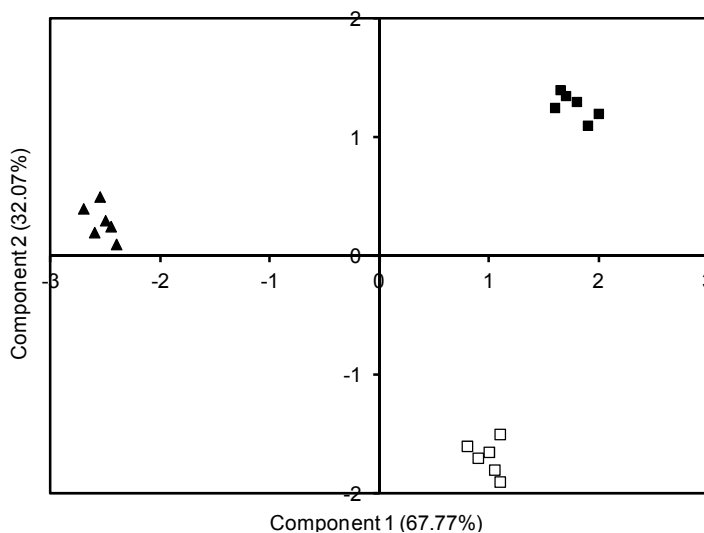


Fig. 7. PCA of volatile fractions of the substances A.1 (□), C.2 (▲) and *n*-butanol solution No. 8 (■)

PCA (Fig. 7) revealed a possibility of differentiation between the samples A.1, C.2 and *n*- solution No. 8. Comparing the fingerprints of volatile fraction of the solutions A.1 and C.2 (Fig. 8a, b) one can notice their diversification, as well. It is a result of different chemical structure and concentrations of A and C.

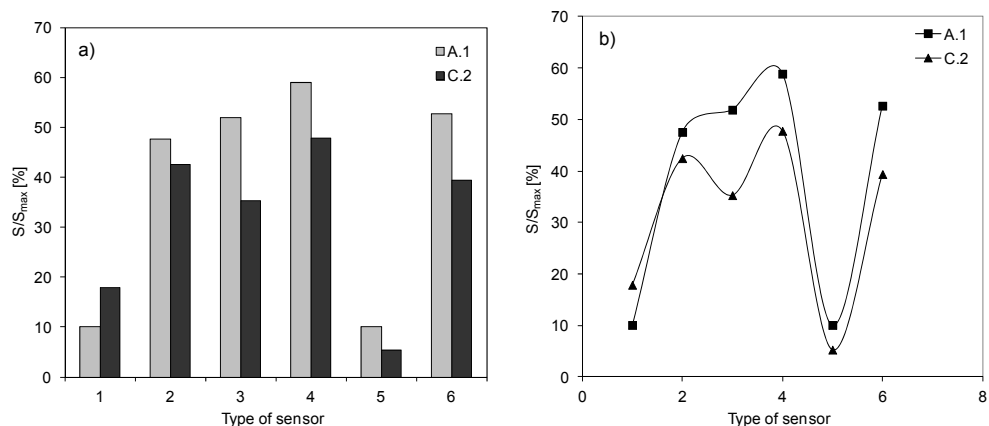


Fig. 8. Mean ratio of the response electronic nose sensors' signal to maximum signal versus sensor type and type of solution: A.1, C.2: a) bar plot, b) linear plot. Type of sensor: 1 – TGS 880, 2 – TGS 825, 3 – TGS 826, 4 – TGS 822, 5 – TGS 2610, 6 – TGS 2602

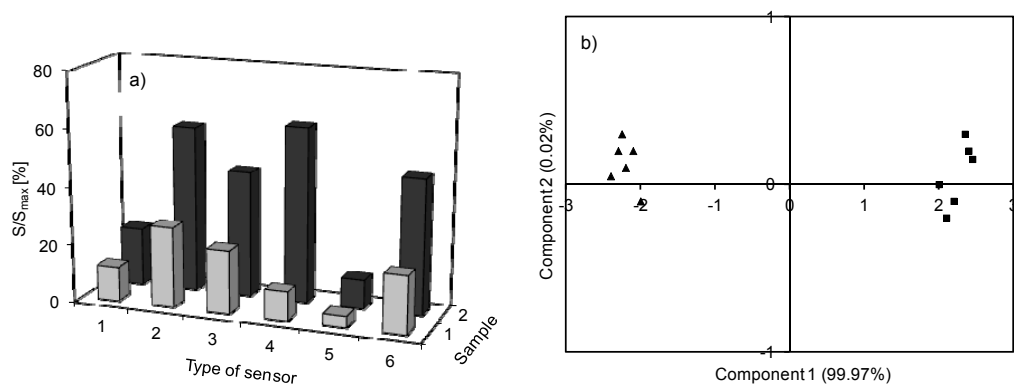


Fig. 9. Mean ratio of the response electronic nose sensors' signal to maximum signal versus sensor type and type of solution (a): C.1 (bright bars)–ABC.2 (dark bars). Type of sensor: 1 – TGS 880, 2 – TGS 825, 3 – TGS 826, 4 – TGS 822, 5 – TGS 2610, 6 – TGS 2602. PCA of volatile fractions of the substances C.1 (■), ABC.2 (▲) (b)

Odor intensity of the samples C.1 and ABC.2 identified by the volunteers was at the level of 1.5 of the reference scale, so both solutions could not be discriminated with the sensory analysis. Fig. 9a presents three-dimensional aroma profiles obtained for C.1 and ABC.2 solutions. Some differences can be observed, which allow differentiation between the solutions. PCA performed (Fig. 9b) confirms such possibility.

Both analysis of three-dimensional aroma profiles as well as PCA confirm that application of the electronic nose equipped with a suitable identification system makes it possible to differentiate between the compounds and their mixtures, which at that particular concentration level cannot be discriminated with a human nose.

Repeatability of the results obtained with the e-nose prototype was at the level of 4.8% *CV*, whereas for the classical sensory analysis it amounted 27% *CV*.

The other, similar measurement series carried out after two month break revealed high convergence with the previous results. Repeatability of the results of the second series obtained with the e-nose prototype was at the level of 5.5% *CV*, whereas for the classical sensory analysis it was 24% *CV*.

5. SUMMARY

Odor analysis is undoubtedly one of the most challenging tasks for analytical chemistry. A number factors hinder correct classical sensory analysis and application of chromatographic techniques. A recent interest in the electronic nose technique, chemical sensors and chemometric data analysis contributes to increasingly wider application of these in everyday life.

Two methods of odor analysis have been presented in this paper. The first one, employing a classical sensory analysis, has been carried out by a trained team of volunteers and has not made it possible to differentiate between aqueous solutions of 1-propanol, benzaldehyde, 2,3-butanedione and their mixtures. There were two reasons of this failure: too high individual smell perceptibility threshold level of the test participants revealed for selected concentration levels of the solutions; in some cases perception of similar odor intensity by the test participants. The solutions A.3, B.2, B.3, C.3 have not been detected by the volunteers, whereas the solutions A.1, C.2 and C.1, ABC.2 were characterized by a similar scale of odor intensity. Application of the electronic nose technique enabled differentiation of the aforementioned solutions. Duration of a single analysis performed with the use of the prototype of electronic nose was relatively short, only 30 s. Repeatability of the results was 4.8–5.5% *CV*, whereas in case of the classical sensory analysis it was at the level 24–27% *CV*. The electronic nose exhibited much higher sensitivity, reproducibility and reliability with respect to the results as compared to the classical sensory analysis.

The authors of the paper believe that optimization of the operation parameters of the prototype of electronic nose will make it possible to differentiate more complicated gaseous mixtures at the concentration level below 50 ppb.

Despite promising results showing superiority of the e-nose technique with respect to the sensory analysis in some circumstances, it must be emphasized that the e-nose can only be a tool supplementary for biological sense of smell. Natural sense still possesses significant excess of receptors (sensors) as compared to the e-nose and its memory capacity (brain) is much higher than the e-nose database. However, in some cases human sense of smell can be insensitive to certain substances, which is the incentive for investigations and development of the e-nose systems.



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