Biosensors and Bioelectronics, Volume 19, Issue 12, 15 July 2004, Pages 1581-1590

Monitoring Haemodialysis Using Electronic Nose and Chemometrics

Reinhard FEND[1], Conrad BESSANT[2], Anthony J. WILLIAMS[3], and Anthony C. WOODMAN[1,*]

1) Cranfield BioMedical Centre, Cranfield University at Silsoe, Silsoe, Bedfordshire, MK 45 4DT, UK
2) Cranfield Centre for Analytical Science, Cranfield University at Silsoe, Bedfordshire, MK 45 4DT, UK
3) Gloucester Royal Hospital, Great Western Road, Gloucester, Gloucestershire, GL 1 3NN, UK

* Corresponding author: Tel.: +44-1525-863517, fax: +44-1525-863540
E-mail address: a.c.woodman@cranfield.ac.uk (A. C. Woodman)

Abstract

An ever-increasing number of patients have to undergo regular renal dialysis to compensate for acute or chronic renal failure. The adequacy of the treatment has a profound effect on patients' morbidity and mortality. Therefore it is necessary to assess the delivered dialysis dose. For the quantification of the dialysis dose two parameters are most commonly used, namely the Kt/V value (normalised dose of dialysis) and the urea reduction rate, yet the prescribed dialysis dose often differs from the actual delivered dialysis dose. Currently, no interactive process is available to ensure optimal treatment. The aim of this study was to investigate the potential for an "electronic nose" as a novel monitoring tool for haemodialysis. Blood samples were analysed using an electronic nose, comprising an array of 14 conducting polymer sensors, and compared to traditional biochemistry. Principal component analysis and hierarchical cluster analysis were applied to evaluate the data, and demonstrated the ability to distinguish between pre-dialysis blood from post-dialysis blood independent of the method used. It is concluded that the electronic nose is capable of discriminating pre-dialysis from post-dialysis blood and hence, together with an appropriate classification model, suitable for on-line monitoring.

Keywords: electronic nose, conducting polymers, renal failure, kidney disease, principal component analysis, hierarchical cluster analysis

1. Introduction

An ever-increasing number of patients have to undergo regular renal dialysis to compensate for acute or chronic renal failure. The estimated annual rate of patients starting renal replacement therapy (RRT) in England and Wales is 89 per million population indicating that approximately 5350 patients started RRT in 2000, haemodialysis (HD) being the predominant form of RRT (UK Renal Registry Report, 2001). The frequency and length of dialysis is generally determined empirically, with little or no on-line analysis available to ensure optimal dosage.

1.1 Renal failure and haemodialysis

Renal failure is characterised by an impaired kidney function leading to disturbances in waste excretion, hormone production and in the regulation of fluid and electrolyte status. Therefore it is necessary to replace the kidney function by means of an artificial device (dialysis) or by organ transplantation. However, artificial devices can only replace certain functions of the kidney, in particular the removal of waste products and excessive body fluids.

It is well known that the prescribed dialysis dose differs from the actually delivered dialysis dose (Manzoni *et al.*, 1996; Keshaviah, 2002). Therefore, it is necessary to quantify the delivered dialysis dose because the adequacy of the dose has a profound effect on patient morbidity and mortality (Manzoni *et al*., 1996; Shak, 1999). The concept of dialysis adequacy was developed in the early 1970s to assess the treatment efficiency of end-stage renal disease (ESRD) patients (Canaud *et al.*, 2000). From the very beginning the removal of small molecules was considered important as they were directly linked to the symptoms and signs of uraemia (Keshaviah, 2002; Lindsay and Sternby, 2001). Therefore, urea has been suggested as a surrogate marker for small toxic solutes (Keshaviah, 2002), and gained wide acceptance in 1975 as a marker substance (Vanholder *et al.,* 1994).

For the quantification of the dialysis dose two parameters are most commonly used, namely the urea reduction rate (URR) and the normalised dose of dialysis (Kt/V) (Lowrie, 2000; Lindsay and Sternby, 2001). Both parameters are based on urea. The URR is calculated from the pre-dialysis and post-dialysis blood urea nitrogen value, whereas the Kt/V value is derived from urea kinetic modelling (Levy

et al., 2001, Kemp *et al.*, 2001). Devices such as the Baxter Biostat 1000 (Baxter Healthcare Corp., McGaw Park, IL), the DQM 200 – Device (Gambro Lund AB, Lund, Sweden), the Bio Care Device (Bio-Care Corp., Hsinchu, Taiwan) and the Bellco – device (Bellco SpA, Miranda, Italy) are commercially available and calculate either of the two parameters on-line. However, there is a controversial discussion about whether these parameters are appropriate or not (Vanholder *et al.,* 2002; Lowrie 2000). However, it has been shown that oversimplification of the "dialysis dose" to the Kt/V index might lead to dramatic underdialysis (Canaud *et al*., 2000). The Kt/V value assesses only the removal of small water-soluble compounds from the body (Vanholder *et al.,* 2002). Hence, the molecular size of urea means that convective and/or diffusive transport of larger molecules is unlikely to be described by urea kinetics (Vanholder *et al.*, 2002; Lowrie, 2000). Both values (Kt/V and URR) assume a dialysis frequency of three times per week. However, recently published data suggest that 5 % of all American patients skip a dialysis session in any month leading to an incorrect Kt/V or URR value (De Palma *et al.,* 2001). Other pitfalls in calculating the Kt/V value come from the incorrect post-dialysis blood sampling such as urea-rebound effect, sample dilution with dialysis fluid or blood recirculation. (De Palma *et al.,* 2001). However, careful post-dialysis blood sampling minimises the influence of these problems (Levy *et al.* 2001).

1.2 Electronic nose technology

Electronic nose (EN) is the colloquial name for an instrument made up of chemical sensors combined with some sort of pattern recognition system (Gardner and Bartlett, 1994). The key idea of an EN is to mimic the human olfactory system. The human olfactory receptors are replaced by chemical sensors, which produce an electrical signal (similar to nerve cells). These signals are subsequently analysed by an appropriate pattern recognition engine. The pattern recognition software corresponds to the cerebral cortex of the brain, and is able to classify or memorise odours. (Bartlett *et al*., 1997; Pearce, 1997 (a and b)).

The most commonly used types of sensors in electronic noses are: metal oxide sensors (Persaud *et al*., 1997; Dickinson *et al*., 1998), conducting polymers (Hatfield *et al*., 1994; Gardner and Bartlett, 1995), and piezoelectric based sensors such as bulk acoustic wave sensors or surface acoustic wave sensors (D'Amico *et al.,* 1997; Chang

et al., 2000). All types of sensors share a common basic principle. The interaction of volatile compounds with the sensor surface leads to a change of physical properties (conductivity, resistance, frequency) of the sensor, which is measured. In other words, the different types of sensor vary in the way the sensor response is generated.

Electronic noses have been applied in several areas to characterise the odour of products such as beer (Pearce *et al*., 1993), boartaint (Bourronnet *et al*., 1995), cardboard papers (Holmberg *et al*., 1995), coffee (Singh *et al*., 1996), and wine (Di Natale *et al*., 1996; Guadarrama *et al*., 2001). More recently electronic noses have been used for the quality control and process monitoring of foodstuffs such as olive oil (Guadarrama *et al*., 2000; Guadarrama *et al*., 2001) or milk spoilage (Magan *et al*, 2001). However, the ability of ENs as a diagnostic tool is attracting more and more research groups. It is well known that smell can be used to diagnose diseases and has been used by both the Greeks and the Chinese since 2,000 BC (Mitruka, 1975). Of particular interest are infectious diseases such as bacterial vaginosis (Chandiok *et al*., 1997), pulmonary infections (Hanson *et al*., 1997), tuberculosis (Pavlou and Turner, 2000), bacteruria (Aathithan *et al*., 2001) and urinary tract infections (Pavlou *et al*., 2002) as well as breath analyses of patients suffering from diabetes (Ping *et al*., 1997) or uraemia (Lin *et al*., 2001). Di Natale and co-workers (1999) investigated the capability of an EN to detect traces of blood in urine samples of kidney patients. However, this group could not give an explanation of which volatiles are responsible for the sensor response.

1.3 Aims

The study was divided into two parts. In the first part, the potential of an electronic nose as a novel monitoring system for haemodialysis was investigated. In the second part, the electronic nose data were compared to traditional biochemical monitoring parameters (urea, creatinine, etc.). The aim of the study was to find out whether there was sufficient information within the data to discriminate between pre and post dialysis blood. This was achieved using exploratory data analysis techniques.

2. Materials and Methods

2.1 Blood Collection

Blood samples were collected either from patients undergoing intermittent HD-treatment at Gloucester Royal Hospital or healthy volunteers (Control blood). Ethical approval for this study was obtained from the Gloucestershire Research Ethics Committee (Study No.: 01/149G). All patients and volunteers participating in this study gave informed written consent before blood samples were taken. Each sample was encoded to ensure anonymity.

All patients are treated either with a Fresenius F6 or Fresenius F8 dialyser (Fresenius AG, Bad Homburg v.d.H., Germany) The surface area of the polysulfone membrane is either 1.3 m^2 (F6 dialyser) or 1.8 m^2 (F8 dialyser). The blood flow varied between 200 and 350 ml/min, depending on the patient, whereas the dialysate flow rate was kept constant at 500 ml/min. All patients included in this study undergo haemodialysis treatment three times a week for four hours per session.

2.1.1 Blood collection for biochemical analysis

From each patient, 10 ml of pre- and post-dialysis blood, respectively and from each volunteer 10 ml of control were drawn and placed in Vacuette® Serum tubes (Greiner, UK) prior to analysis by the Department of Chemical Pathology at Gloucestershire Royal Hospital (Gloucester, UK).
The relevant details (Gender, Age) are summarised in table 1.

Table 1

2.1.2 Blood collection for the analysis of volatile compounds using an electronic nose

The analysis of volatile compounds by means of an electronic nose was performed on whole blood. Therefore, sampling tubes containing EDTA as an anticoagulant (Greiner bio-one, Vacuette®, coagulation tubes) were used for blood sampling. A subset (8 control samples, 11 dialysis patient) from the original 11 volunteers and 28

patients, respectively were used for this experiment, i.e. the blood samples were taken at the same time and from the same patients as for the biochemical analysis.

. From each patient, 4 ml of pre- and post-dialysis blood, respectively and from each volunteer 4 ml of control blood were taken. The blood samples were stored at – 20 ºC until the analysis was performed.

The relevant details (Gender, Age) are summarised in table 2.

Table 2

2.2 Analysis of volatile compounds in blood

2.2.1 Preparation of the blood samples

Blood samples were defrosted on ice to minimise the loss of volatile compounds. Samples were diluted 1:4 in 0.9 % (w/v) NaCl-solution and mixed thoroughly. A 1 ml aliquot of the diluted blood was transferred into a 10 ml headspace vial (Jaytee Bioscience Ltd., UK) and immediately sealed using a crimp cap with Silicon/Teflon-septum (Jaytee Bioscience Ltd, UK). The sealed headspace vials were subsequently incubated for 45 minutes at 37 ºC.

2.2.2 Gas-sensing system and headspace sampling

For this study an EN (Bloodhound BH-114, Bloodhound Sensors, Leeds, UK) employing 14 conducting polymers based on polyaniline was used. The sensor unit automatically sets two calibration points. The first one is the baseline, which is obtained when activated carbon filtered (Carbon Cap 150, Whatman) air is passed over the sensor at a flow rate of 4 ml min^{-1}. The second calibration point is a reference point obtained from the headspace of a control sample vial containing 9 ml of RO-water (Fig. 1).

Two sensor parameters were selected in this study: divergence (maximum step response) and area (area under the response curve). The sampling profile was set at 10 s of absorption and 15 s of desorption.

For the analysis of the unknown headspace, the sample vials were connected to the "electronic nose" by inserting a needle into the headspace of the sample vials (Fig. 1). The test gaseous phase was passed over the sensor surface at a flow rate of 200 ml min^{-1}, which was automatically set by the sensor unit. Between each measurement, a time delay of 2 minutes was set. The individual samples were randomly analysed.

Fig. 1

2.3 Data Analysis

To analysis the multivariate data, two standard methods, namely principal component analysis (PCA) and hierarchical cluster analysis (HCA) were applied. Prior to these methods no scaling was undertaken. To perform these analyses an Excel add-in software (*XL*stat©, version 3.4, France) was used.

2.3.1 Principal Component Analysis

PCA is an unsupervised data reduction method. The basic idea of the method is to describe the variation of a multivariate data set in terms of a set of uncorrelated variables, each of which is a particular linear combination of the original variables. In other words, the original data matrix is projected from a high dimensional space into a less dimensional space, preferably a plane or a 3-dimensional space. During the process the original data set is reduced in dimensions, i.e. is compressed, with as little loss of information as possible. This is achieved by filtering out the noise in the original data matrix, without removing essential information described in the variance of the data [Otto, 1999; Massart *et al*., 1988].

Mathematically, the key idea of PCA is to decompose the original *i x j* data matrix ***X*** into its *i x k* score matrix ***T***, its *k x j* loading matrix ***P*** and the residual matrix ***E*** according to:

$$\boldsymbol{X = TP + E} \quad (1)$$

where *i* is the number of samples, *j* is the number of variables and *k* is the number of principal components (PCs). The PCs are determined on the basis of the maximum variance criterion. Each subsequent PC describes a maximum of variance, which is not modelled by the previous one. According to this, the first PC contains the most of the variance of the data [Otto, 1999; Everitt and Dunn, 2001]. The relationship between samples can be visualised by plotting the scores against each other.

2.3.2 Hierarchical Cluster Analysis

HCA is an unsupervised method that combines individual samples into clusters according to their similarities to each other. The similarity between two samples is determined by the (Euclidean) distance between them, which can be calculated for two samples p_1 (x_1, y_1) and p_2 (x_2, y_2) as follows:

$$d_{(p1,p2)} = [(x_1 - x_2)^2 + (y_1 - y_2)^2]^{1/2} \quad (2)$$

where $d_{(p1,p2)}$ is the distance between the two samples.

The next step is the reduction of the distance matrix by aggregation of clusters, whereby the clusters with the shortest distances between them are aggregated first. The most commonly used technique, and the one used here, is the Ward's method. The key idea of this method is that clusters are aggregated in such a way, that a minimum increase in the within-group variance results [Otto, 1999; Everitt and Dunn, 2001].

3. Results

3.1 Biochemical Analysis

Five biochemical parameters (urea, creatinine, carbon dioxide, phosphate, and calcium phosphate) were selected for this analysis and are summarised in table 3.

Table 3

As expected, small uremic toxins such as urea and creatinine were effectively removed during haemodialysis. However, the post-dialysis concentrations of these molecules were higher than compared to healthy subjects. These results are well known, but it is almost impossible to present this multivariate data set in a reasonable way (two- or three-dimensional space) in their raw form. Therefore, the original matrix was reduced in dimensionality using PCA. The results of plotting the PCA scores for all samples is shown in Fig 2.

Fig. 2

Fig 2a demonstrates that it is possible to discriminate between post- and pre-dialysis blood (dashed line) by using the first two PCs (axis 1 and 2), which account for 83 % of the variance of the original data matrix. However, it was impossible to separate control blood from post-dialysis blood, but according to table 3, there is a notable difference. After introducing a third PC (vertical axis 3, Fig. 2b), which accounts for 14 % of variance, it was possible to distinguish between control blood and post-dialysis blood.

The HCA revealded similar results to the PCA, as shown in Fig. 3. From the original 28-dialysis patients used in this analysis, only in 3 cases (patients: 15, 19, and 24) was it not possible to distinguish between post- and pre-dialysis blood. This is due to small difference between pre – and post-dialysis blood, i.e. there is just a slight decrease of the concentrations of the five biochemical parameters during dialysis treatment (data not shown).

Fig. 3

3.2 Analysis of volatile compounds in blood using an electronic nose

As can be seen in Fig. 4, a good separation between control blood, pre- and post-dialysis blood was obtained (dashed lines added by the author). The first two principal components (PC) account for 94 % of the variance in the original data matrix. The third PC accounts for just 2 % of the variance, and therefore made no significant contribution to the discrimination of the samples.

Fig. 4

Similar results were obtained when using HCA, as shown in Fig. 5. In one case (patient 9), no difference between pre- and post-dialysis blood was observable. It can be seen that the odour of the post-dialysis sample of patient 1 (PoD1) resembled the odour of control blood, and the odour of the pre-dialysis sample (PD1) was similar to post-dialysis blood.

Fig. 5

4. Discussion

This study has shown that by application of multivariate methods combined with traditional biochemistry (see 3.1) and modern electronic nose technology (see 3.2), respectively, it might be possible to discriminate “uremic” blood from control blood. However, more important is the possibility to distinguish between pre- and post-dialysis blood. It has to be mentioned, that PCA as well as HCA are exploratory data analysis techniques, whose main goal to visualise the original data matrix, i.e. they are not classification methods. The application of these methods to traditional biochemical data (Fig. 2 and 3), demonstrated the efficacy of these tools, even when the outcome was not “blinded”.

In the second part of the study, an electronic nose was applied to investigate the headspace of "uremic" blood and control blood, respectively. The results of this experiment were similar to the first one, i.e. a good separation between the different types of blood was possible. However, the physical and chemical basis leading to the sensor response were more complex. An electronic nose is an array of chemical gas sensors, therefore only gas molecules in the sample headspace contribute to the sensor response. The volatility of molecules is influenced by many parameters such as concentration, equilibration temperature, equilibration time, and viscosity of the sample (Seto, 1994).

To determine which volatiles may be responsible for the difference in electronic nose response, it is important to consider the nature of chronic renal failure and its treatment. Chronic renal failure is characterised by the accumulation of waste products and excessive body fluids. These metabolic waste products as well as body fluids are normally excreted with urine. Therefore, "uraemic" blood contains numerous compounds, some of them are volatile others are not. In the past, many groups tried to identify "uraemic" molecules, which are generally divided into three groups: small, middle and large molecules (Levy et al. 2001). Volatile compounds are generally small and polar (Gardner and Bartlett, 1999). In the past, several research groups identified these molecules using mainly gas chromatography and gas chromatography – mass spectroscopy. The range of molecules retained in uraemia is broad, including free organic acids, phenolic compounds, aliphatic amines, alcohols, aldehydes, ketones and guanidines (Baba *et al*., 1984; Liebich *et al.,* 1984; Niwa *et al.,* 1981; Liebich *et al.,* 1977). All of them are present in elevated concentrations in CRF patients. Bowen *et al*. (1975) investigated benzyl alcohol present in blood of CRF patients. They found elevated values of benzyl alcohol in dialysis patients before the session and lower levels after the treatment. Benzyl alcohol was not found in normal controls. Baba *et al.* (1984) analysed serum aliphatic amines using HPLC. They found a six-fold increase of methylamine and dimethylamine in uraemic serum compared to normal control subjects. The degree of removal of these compounds is smaller than for urea and creatinine and is approximately 55% (Baba *et al*, 1984; Lichtenberger *et al*., 1993). The same group found similar concentration of the volatile ethanolamine in pre-dialysis serum and control serum, but a two-fold increased level in post-dialysis samples (Baba *et al*., 1984). Other investigators found volatiles such as methylmercaptan (Dowty *et al*., 1975), acetone, 2-butanone,

chloroform, benzene, toluene, pyridine, dipropylketone, cycloheaxanone, and 4-heptanone in uraemic blood (Liebich, 1977). All these substances are characterised by a relatively small molecular weight (M_r < 500) and are retained in CRF patients. However, due to their low molecular weight they are effectively removed during a haemodialysis session, leading to a decreased concentration in post-dialysis samples. On the other hand, investigators found an increased oxidative stress in HD patients during the treatment mainly caused by the incompatibility of the dialysis membrane. This additional oxidative stress leads to the generation of free radicals causing enhanced lipid peroxidation (Tetta *et al.,* 1999; Erdogan *et al*., 2002). The end products of lipid peroxidation are aldehydes (malonaldehyde, propanal), ketones (acetone) and small carbohydrates (ethane, pentane) (Hageman *et al.,* 1992; Capodicasa *et al.*, 1999). This process leads to a continuous increase of volatiles in the blood during the treatment.

From this, we conclude that the sensor response is caused by the combined effect of a) removal of accumulated waste products, b) the generation of volatiles during HD via lipid peroxidation and c) probably the generation of volatiles during the sample incubation period (e.g. ammonia from urea). Therefore, the difference between pre- and post-dialysis samples is mainly caused by different concentrations of molecules rather than the appearance of new molecules. Hudon *et al*. (2000) found that conducting polymers show a linear relationship between sensor response and concentration. This finding is in agreement with Persaud *et al.* (1996). Our hypothesis is supported by the fact, that conducting polymers are very sensitive (5 – 0.1 ppm) towards polar molecules (Schiffman *et al*. 1997).

In the past only a few applications have been described in the literature where electronic noses have been used for renal diseases. Among these is the work of Lin *et al.* (2001), who applied an electronic nose for the diagnosis of uraemia. This group found marker substances in the breath of chronic renal failure patients such as di- and trimethylamine, which allowed the differentiation between healthy controls and uraemic patients. Based on these findings, breath analysis may be a useful substrate for a new monitoring system.

The main aim was to investigate the potential of an electronic nose as a novel on-line monitoring system for haemodialysis. To date, no medical application could be found in the literature where a gas-sensing array was applied on-line and in real-time. Such applications described in the literature are mainly located in the food industry or

for waste water treatment. For example, Bourgeois *et al.* (2001) used an electronic nose for the on-line monitoring of waste water streams.

The electronic nose itself showed good reproducibility, i.e. any sensor drift was insignificant compared to the difference between samples types.

5. Conclusions

This study suggests that electronic nose technology might be a useful tool in discriminating pre-dialysis form post-dialysis blood as well as control blood. Together with an appropriate classification model, it might be possible to build an on-line monitoring system for the management of renal failure. It might also be possible to improve and modify currently used sensor arrays towards specific volatile markers or marker groups, which would simplify the optimisation of such applications.

However, more work needs to be done to make this application a rapid and efficient diagnostic/monitoring device.

Acknowledgement

The authors are grateful to the Renal Patient's Association and Fresenius for financial support and to the Public Health Laboratory staff (Gloucester Royal Hospital, UK). R. Fend would like to thank Sharon King for her help.

References

Aathithan S., Plant J. C., Chaudry A. N., French G. L. (2001), Diagnosis of Bacteriuria by Detection of Volatile Organic Compounds in Urine using an Automated Headspace Analyser with Multiple Conducting Polymer sensors, Journal of Clinical Microbiology, 39:2590-2593

Baba S., Watanabe Y., Gejyo F., Arakawa M. (1984), High-performance liquid chromatographic determination of serum aliphatic amines in chronic renal failure, Clinica Chimica Acta 136:49-56

Bartlett P. N., Elliot J. M., and Gardner J. W. (1997), Application of, and Developments in, Machine Olfaction, Annali Di Chimica, 87:33-44

Bourgeois W., Burgess J. E., Stuetz R M. (2001), On-line monitoring of waste water quality: a review, J. Chem. Technol. Biotechnol. 76:337-348

Bourronnet B *et al*. (1995), Application of a multi-gas-sensor device in the meat industry for boar-taint detection, Sensors and Actuators B, 26-27:250-254

Bowen D., Cowburn D., Rennekamp M., Sullivan J. (1975), Benzyl alcohol: high levels found in plasma of uremic patients on hemodialysis, Clinica Chimica Acta 61:399-401

Canaud B., Bosc J. Y., Carrol L., *et al*. (2000), Urea as a marker of adequacy in hemodialysis: Lessons from in vivo urea dynamics monitoring, Kidney Internation, 58:28-40

Capodicasa E., Trovarelli G., De Medio G., Pelli M., Lippi G., Verdura C., Timio M. (1999), Volatile Alkanes and Increased Concentrations of Isoprene in Exhaled Air during Hemodialysis, Nephron 82:331-337

Chandiok S., Crawley B. A., Oppenheim B. A., *et al*. (1997), Screening for bacterial vaginosis: a novel application of artificial nose technology, Journal of Clinical Pathology, 50:790-795

Chang S. M., Muramatsu H., Nakamura C. *et al*. (2000), The principle and applications of piezoelectric crystal sensors, Materials Sciences and Engineering C, 12:111-123

D'Amico A., Di Natale C. and Verona E. (1997), Acoustic Devices in *Handbook of Biosensors and Electronic Noses Medicine, Food and the Environment*, edited by E. Kress-Rogers, CRC press, New York, pp 197-223

De Palma J. R., Pittard J. D. (2001), Dialysis dose, www.hemodialysis-inc.com

Dickinson T. A., White J., Kauer J. S., Walt D. R. (1998), Current trends in artificial-nose technology, Tibtech 16:250-258

Di Natale C. *et al*. (1996), An electronic nose for the recognition of the vineyard of a red wine, Sensors and Actuators, B33:83-88

Di Natale C., Mantini A., Macagnano A., *et al*. (1999), Electronic nose analysis of urine samples containing blood, Physiological Measurements, 20:377-384

Dowty B., Charlisle D., Laseter J., Gonzalez F. (1975), Gas chromatographic mass spectrometric computer analysis of volatile compounds in blood plasma from hemodialysis patients, Biomed. Mass. Spectrometry 2:142-147

Erdogan C. *et al*. (2002), The evaluation of oxidative stress in patients with chronic renal failure, Clinica Chimca Acta 322:157-161

Everitt B. S., Dunn G. (2001), Applied Multivariate Data Analysis, Arnold Publishers, London, 2001

Gardner J. W., Bartlett P. N. (1995), Application of conducting polymer technology in microsystems, Sensors and Actuators A, 51:57-66

Gardner J. W., Bartlett P. N. (1994), A brief history of electronic noses, Sensors and Actuators B, 18-19:211-220

Gardner J. W, Bartlett P. N. (1999), Electronic Noses: Principles and Applications, Oxford University Press, Oxford, 1999

Guadarrama A., Rodriguez-Mendez M. L., De Saja J. A., *et al*. (2000), Array of sensors based on conducting polymers for the quality control of the aroma of the virgin olive oil, Sensors and Actuators B, 69:276-282

Guadarrama A., Rodriguez-Mendez M. L., Sanz C., *et al*. (2001), Electronic nose based on conducting polymers for the quality control of the olive oil aroma. Discrimination of quality, variety of olive and geographic origin, Analytica Chimica Acta, 342:283-292

Hageman J., Bast A., Vermulen N. (1992), Monitoring of oxidative free radical damage in vivo: analytical aspects, Chem. Biol. Interactions, 82:243-293

Hanson C. W., *et al*. (1997), The use of a novel electronic nose to diagnose the presence of intrapulmonary infection, Anaesthesiology, 87 (3A), 269.

Hatfield J. V., Neaves P., Hicks P. J., *et al*. (1994), Towards an integrated electronic nose using conducting polymer sensors, Sensors and Actuators B, 18-19:221-228

Holmberg M., Winquist F., Lundström I., *et al*. (1995), Identification of paper quality using a hybrid electronic nose, Sensors and Actuators B, 26-27:246-249

Hudon G., Guy C., Hermia J. (2000), Measurement of Odor Intensity by an Electronic Nose, Air and Waste Management Association, 50:1750-1758

Kemp H. J., Parnham A., Tomson C. R. (2001), Urea kinetic modelling: a measure of dialysis adequacy, Annals of Clinical Biochemistry 38:20-27

Keshaviah P. (2002), Adequacy of dialysis: Comparison of hemodialysis (HD) to CAPD, Indian Journal of Nephrology, 16:51-55

Levy J., Morgan J., Brown E. (2001), Oxford Handbook of Dialysis, Oxford University Press, Oxford, 2001

Lichtenberger L., Gardner J., Barreto J., Dial E., Weinman E. (1993), Accumulation of Aliphatic Amines in Gastric Juice of Acute Renal Failure Patients, Digestive Diseases and Sciences 38/10:1885-1888

Lieblich H., Woell J. (1977), Volatile substances in blood serum: profile analysis and quantitative determination, Journal of Chromatography, 142:505-516

Lieblich H, Pickert A., Tetschner B. (1984), Gas chromatographic and gas chromatographic-mass spectrometric analysis of organic acids in plasma of patients with chronic renal failure, Journal of Chromatography 289:259-266

Lin Y. J., Guo H., Chang Y. (2001), Application of the electronic nose for uremia diagnosis, Sensors and Actuators B, 76:177-180

Lindsay R. M. and Sternby J. (2001), Future Directions in Dialysis Quantification, Seminars in Dialysis 14:300-307

Lowrie E. G. (2000), The Normalised Treatment Ratio (Kt/V) Is Not the Best Dialysis Dose Parameter, Blood Purification, 18:286-294

Magan N., Pavlou A., Chrysanthakis I. (2001), Milk-sense: a volatile sensing system recognises spoilage bacteria and yeasts in milk, Sensors and Actuators B, 72:28-34

Manzoni C., Di Filippo S., Corti M, *et al*. (1996), Ionic dialysance as a method for the on-line monitoring of delivered dialysis without blood sampling, Nephrology Dialsysis Transplantation 11:2023-2030

Massart D. L., Vandeginste B. G. M., Dernign S. N., et al. (1988), Chemometrics: A Textbook. Elsevier Science Publishers, 1998

Mitruka B. M., (1975), Presumptive diagnosis of infectious diseases, in: Mitruka B. M. (ed.). *Gas chromatographic applications in microbiology and medicine*, New York, John Wiley and Sons, pp: 349-374

Niwa T., Maeda K., Ohki T., *et al.* (1981), A gas chromatographic-mass spectrometric analysis for phenols in uremic serum, Clinica Chimica Acta 110:51-57

Otto M. (1999), Chemometrics: Statistics and Computer Application in Analytical Chemistry, Wiley-VCH, 1999

Pavlou A. K., Magan N., McNulty C. *et al*., (2002), Use of an electronic nose system for diagnosis of urinary tract infections, Biosensors and Bioelectronics 17:893-899

Pavlou A., Turner A. P. F. (2000), Sniffing out the Truth: Clinical Diagnosis using the Electronic Nose, Clin Chem Lab Med, 38:99-112

Pearce T. C. (1997a), Computational parallels between the biological olfactory pathway and its analogue 'The electronic nose'. 1. Biological olfaction, Biosystemes, 41:43-67

Pearce T. C. (1997b), Computational parallels between the biological olfactory pathway and its analogue 'The electronic nose'. 2. Sensor based machine olfaction, Biosystemes, 41:69-90

Pearce T. C., Gardner J. W., Friel S. (1993), Electronic nose for monitoring the flavour of beers, Analyst, 118:371-377

Persaud K. C., Khaffaf S. M., Payne J. S., Pisanalli A. M., Lee D. H., Byun H.G., (1996), Sensor array techniques for mimicking the mammalian olfactory system, Sensors and Acutators B 35-36:267-273

Persaud K. C. and Travers P. J. (1997), Arrays of Broad Specificity Film for Sensing Volatile Chemicals, in *Handbook of Biosensors and Electronic Noses Medicine, Food and the Environment*, edited by E. Kress-Rogers, CRC press, New York, pp. 563-592

Ping W., Yi T., Haibao X., *et al*. (1997), A novel method for diabetes diagnosis based on electronic noses, Biosensors and Bioelectronics, 12:1031-1036

Schiffman S., Kermani B. Nagle H. (1997), Analysis of Medication Off-odors Using an Electronic Nose, Chemical Senses 22/2:119-128

Seto Y., (1994) Determination of volatile substances in biological samples by headspace gas chromatography, Journal of Chromatography A, 674:25-62

Shak C. G. (1999), The role of Urea Kinetic Modelling in Determining Adequacy of Hemodialysis, Nephrology News & Issues 13:14-16

Singh P. B., Brown R. E., Roser B. (1996), MHC antigens in urine as olfactory recognition clues. Nature 327:161-164

Tetta C, Biasioli S. Schiavon R., Inguaggiato P., David S., Panichi V., Wratten M. (1999), An Overview of Haemodialysis and Oxidant Stress, Blood Purification 17:1118-126

UK Renal Registry Report (2001), UK Renal Registry, Bristol, UK, eds: Ansell D., Feest T,

Vanholder R., De Smet R., Chen H., *et al*. (1994), Uremic Toxicity: The middle molecule hypothesis revisited, Seminars in Nephrology 14/3:205-218

Vanholder R., De Smet R., Lesaffer G. (2002), Dissociation Between Dialysis Adequacy and Kt/V, Sem in Dialysis 15/1:3-7

Legend:

Table 1: Personal details of volunteers / patients for the biochemical analysis

Table 2: Personal details of volunteers / patients for the analysis of volatile compounds using an electronic nose

Table 3: Summary of results of biochemical analysis, showing minimal (min) and maximum (max) concentration as well as the mean concentration of Control blood, Post- and Pre-dialysis blood

Fig. 1: Experimental set-up of the electronic nose. The set-up consists of the electronic nose itself, a sample and a control vial as well as two activated carbon filters and a HEPA-Vent filter. The carbon filters ensure an odourless airflow over the sensor surface and control and sample headspace, whereas the HEPA-VENT filter prevents the fouling of the sensor surface. The electronic nose is connected to a PC running the control software and a data analysis package.

Fig. 2: PCA – Analysis of Control blood (■), Post-dialysis blood (▲) and Pre-dialysis blood (●) based on five biochemical parameters. (a) The first two principal components allow the discrimination between pre-dialysis blood and a mixed cluster containing post-dialysis blood samples and control blood samples. (b) The introduction of a third principal component allows the discrimination between post-dialysis blood and control blood. The Post- and Pre-dialysis blood with the same number belong to the same patient.

Fig. 3: Cluster analysis of Control blood, Post-dialysis blood and Pre-dialysis blood based on five biochemical parameters Legend: PD = Pre-dialysis blood, PoD = Post-dialysis blood, and C = Control blood

Fig. 4: PCA – Analysis of Control blood (■), Post-dialysis blood (▲) and Pre-dialysis blood (●) after 45 min incubation at 37 ºC. Post- and Pre-dialysis blood with the same number belong to the same patient.

Fig. 5: Cluster Analysis of Control blood, Post- and Pre-dialysis blood after 45 min incubation at 37 ºC. Legend: PD = Pre-dialysis blood, PoD = Post-dialysis blood, and C = Control blood

Table 1

	Volunteers / Patients			*Age*		
	No. of samples	*Female*	*Male*	*Mean*	*Median*	*Range*
Control blood	11	6	5	28.3	27	23 - 37
Pre- and Post-dialysis blood	28	7	21	72.3	72.5	51 - 90

Table 2

	Volunteers / Patients			*Age*		
	No. of samples	*Female*	*Male*	*Mean*	*Median*	*Range*
Control blood	8	5	3	28	26.5	23 - 37
Pre- and Post-dialysis blood	11	3	8	72.2	77	51 - 86

Table 3

		Control blood	Post – dialysis blood	Pre – dialysis blood
Urea [mmol L^{-1}]	Min	3.61	3.0	9.6
	Max	7.37	16.6	36.1
	Mean	4.87	7.45	21.4
Creatinine [mmol L^{-1}]	Min	63.7	183	402
	Max	100.2	659	1194
	Mean	77.2	366.5	788.5
CO_2 [mmol L^{-1}]	Min	23.12	26.0	20.00
	Max	30.38	35.0	31.00
	Mean	25.76	29.8	25.39
Phosphate [mmol L^{-1}]	Min	1.07	0.52	0.79
	Max	1.30	1.65	2.78
	Mean	1.16	0.94	1.64
Ca^{2+} x PO_4^{2-} [mmol L^{-1}]	Min	2.56	1.26	1.90
	Max	3.50	4.31	6.67
	Mean	2.90	2.37	4.17

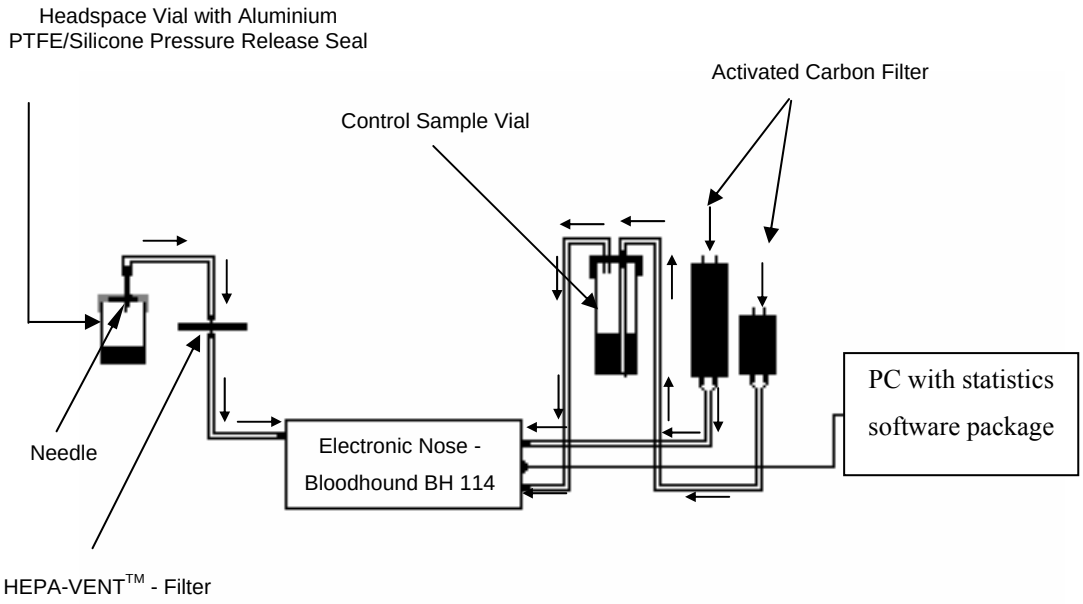
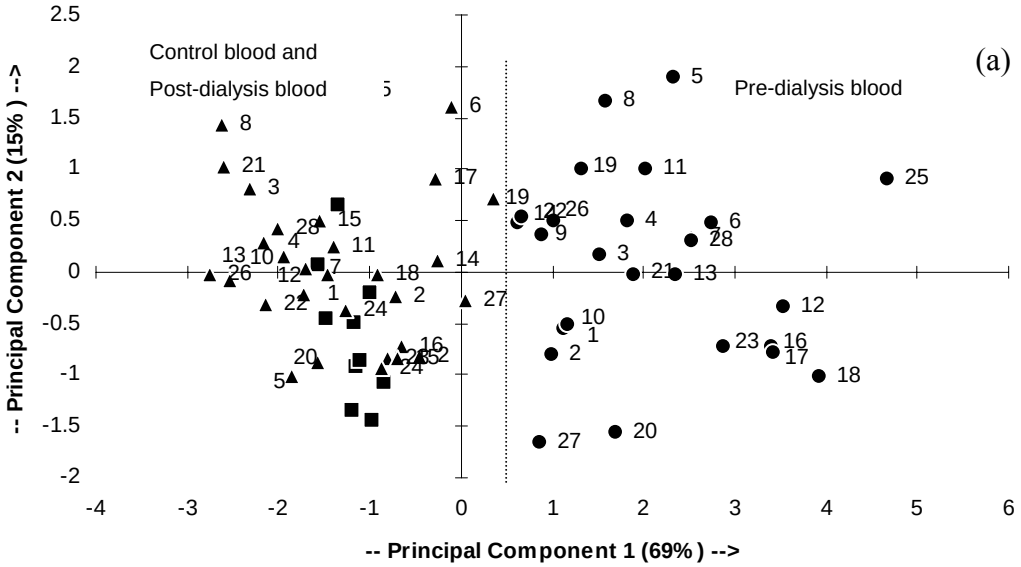

Headspace Vial with Aluminium
PTFE/Silicone Pressure Release Seal
Activated Carbon Filter
Control Sample Vial
PC with statistics
software package
Needle
Electronic Nose -
Bloodhound BH 114
HEPA-VENT™ - Filter

Fig. 1


(a)
Control blood and
Post-dialysis blood
Pre-dialysis blood
-- Principal Component 2 (15%) -->
-- Principal Component 1 (69%) -->

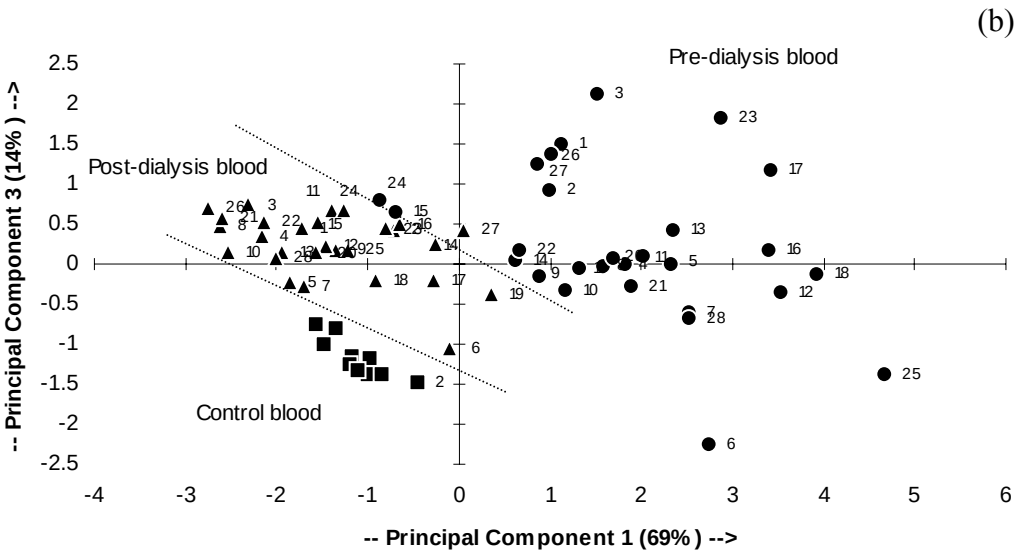

(b)
Pre-dialysis blood
Post-dialysis blood
Control blood
-- Principal Component 3 (14%) -->
-- Principal Component 1 (69%) -->

Fig. 2

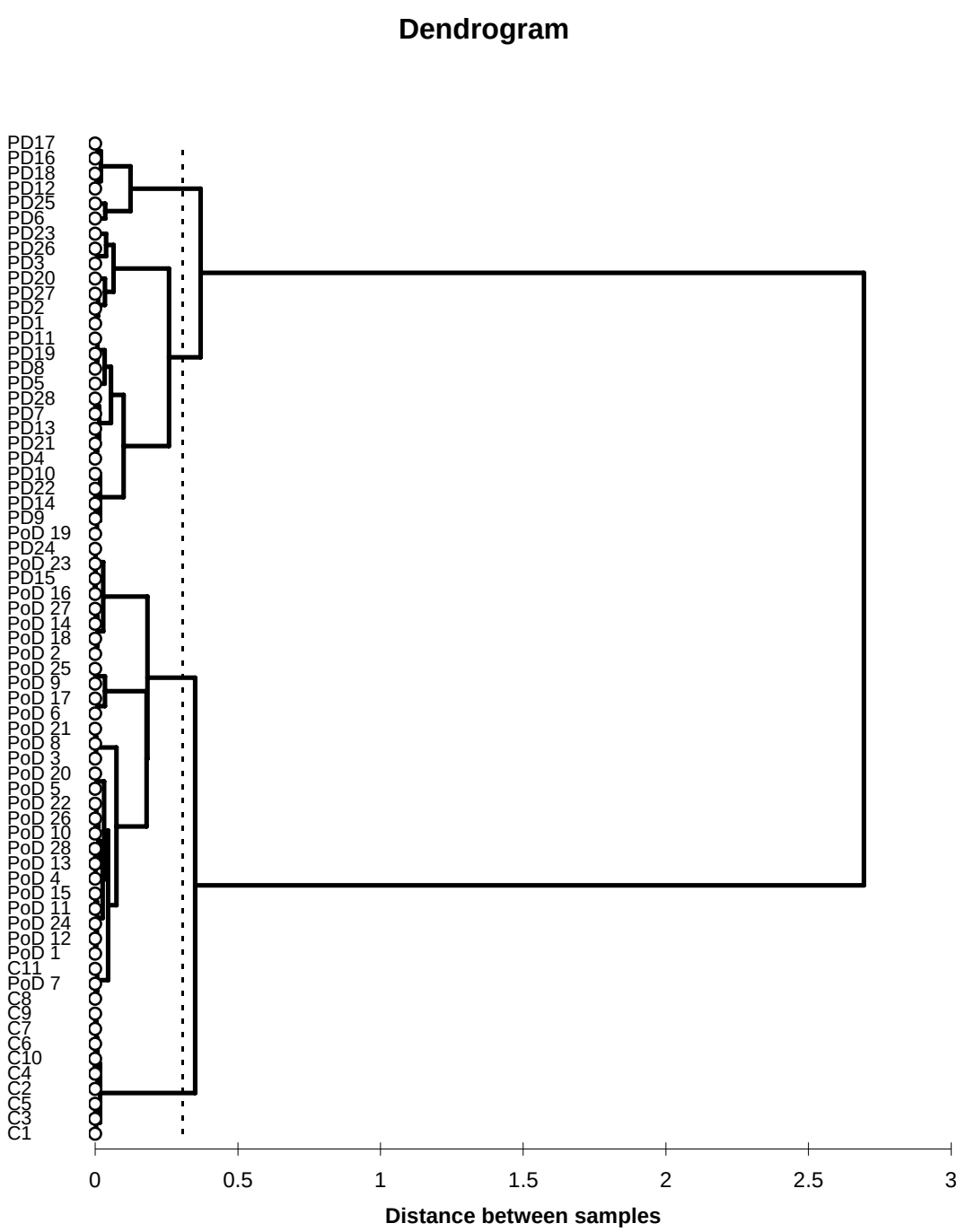

Dendrogram
PD17
PD16
PD18
PD12
PD25
PD6
PD23
PD26
PD3
PD20
PD27
PD2
PD1
PD11
PD19
PD8
PD5
PD28
PD7
PD13
PD21
PD4
PD10
PD22
PD14
PD9
PoD 19
PD24
PoD 23
PD15
PoD 16
PoD 27
PoD 14
PoD 18
PoD 2
PoD 25
PoD 9
PoD 17
PoD 6
PoD 21
PoD 8
PoD 3
PoD 20
PoD 5
PoD 22
PoD 26
PoD 10
PoD 28
PoD 13
PoD 4
PoD 15
PoD 11
PoD 24
PoD 12
PoD 1
C11
PoD 7
C8
C9
C7
C6
C10
C4
C2
C5
C3
C1
0
0.5
1
1.5
2
2.5
3
Distance between samples

Fig. 3

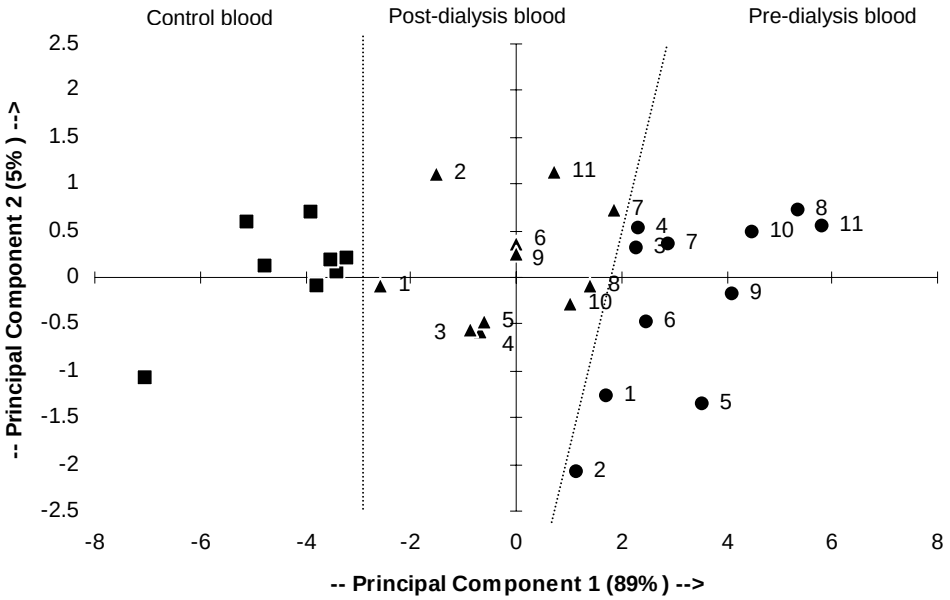

Control blood
Post-dialysis blood
Pre-dialysis blood
-- Principal Component 2 (5%) -->
-- Principal Component 1 (89%) -->

Fig. 4

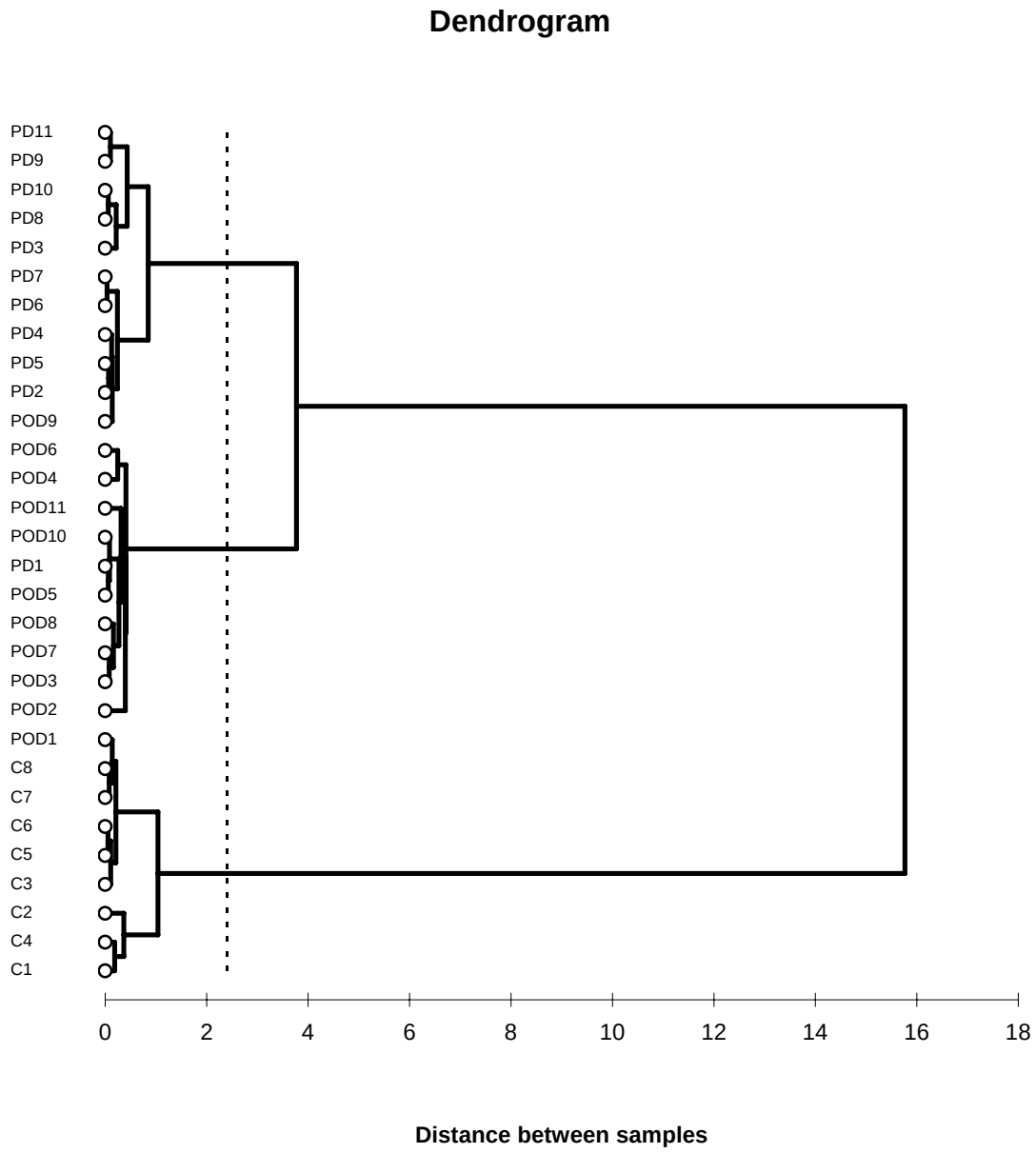

Dendrogram
PD11
PD9
PD10
PD8
PD3
PD7
PD6
PD4
PD5
PD2
POD9
POD6
POD4
POD11
POD10
PD1
POD5
POD8
POD7
POD3
POD2
POD1
C8
C7
C6
C5
C3
C2
C4
C1
0
2
4
6
8
10
12
14
16
18
Distance between samples

Fig. 5