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Towards reliable estimation of an "electronic tongue" predictive ability from PLS regression models in wine analysis

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ABSTRACT

The paper is devoted to an assessment of the predictive power of PLS (partial least squares) models derived from "electronic tongue" data. A multisensor system ("electronic tongue") based on a potentiometric platform was applied to the analysis of wines. Both white and red wine varieties were analyzed employing different sensor arrays. 36 different samples of white wines from New Zealand (Chardonnay, Sauvignon Blanc, Pinot Gris varieties) were analyzed by a number of standard chemical techniques to assess the contents of free and total sulfur dioxide, total acidity, ethanol, pH and some phenolics. Furthermore, 27 samples of red wines produced in Slovakia (Blaufränkisch variety) were assessed by a skilled sensory panel to rate a set of 7 taste descriptors. In addition, all of the wines were analyzed by potentiometric electronic tongue (ET). PLS regression (partial least squares) was used to assess the correlation between ET response, and chemical analytical data, or human perceived sensory characteristics of the wines. Methods that are widely used in the ET literature for estimation of the predictive ability of the PLS models, such as full cross-validation and test set validation with a single random split of samples, were compared with a k-fold random split test set approach. It was shown that the latter does not tend to produce over-optimistic results in small data sets, as are typically available in ET research.

1. Introduction

Comprehensive information on the quality and composition of food products is becoming increasingly important for consumer choice. Geographical origin, agricultural practices and chemical composition, along with sensory qualities play a vital role in the purchase decision of consumers. These factors are even more important when talking about wines. A large variety of different parameters affects the quality and flavour of wine. Despite being extensively studied over the last century, wine chemistry and the relationship between wine composition and flavour is still *terra incognita* in many aspects [1]. From the point of view of wine quality, both chemical composition and sensory description play important roles.

Chemical analysis of wine is a mature field of research and almost every type of modern advanced analytical technique has been applied to wine. Methods such as gas chromatography (GC) [2], high-performance liquid chromatography (HPLC) in different modes [3–5], as well as GC × GC (two dimensional gas chromatography) [6], GC–TOF (gas chromatography–time-of-flight mass-spectrometry) [7], LC–MS/MS (liquid chromatography–two dimensional mass-spectrometry) [8] and nuclear magnetic resonance spectroscopy (NMR) [9] are reported to be appropriate for the quantification of alcohols, organic acids, aroma compounds, phenolics, sulfur-containing compounds, etc., in different types of wines. Though being very precise and allowing highly selective determination of individual compounds in the wine, such as certain polyphenols, these complicated methods are usually extremely expensive, slow, require tedious sample preparation steps and can only be run by highly skilled professionals. In spite of multiple elaborated methods of instrumental analysis, unambiguous and clear correlations between the concentration of selected components, on the one hand, and flavour attributes of the wine on the other hand, can rarely be derived.

A trained sensory panel is the only possible way to evaluate the flavour of wine, and while being arguably the oldest analytical technique in the world, flavour assessment by humans is inevitably associated with a number of serious drawbacks. The most important one is the subjectivity of the assessment; the score for particular sample depends greatly on the physical condition, health and mood of the panellist, even if that person is highly trained which often leads to irreproducible results. Another issue is the fast blocking of human tongue taste receptors during a sensory session, which results in a very limited number of sensory

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assessments per day [10,11]. Both training and preparation of sensory experts are expensive and time-consuming.

The first attempts to apply the electronic tongue (ET) to the wine analysis date back to the late 1990s [12,13], and in recent years there has been a growing interest in the development of sensor systems for food and beverage applications. In the future, such systems are likely to become realistic alternatives for expensive and time-consuming procedures in wine analysis, both chemical and sensory, as simple and fast devices for routine wine quality control. The electronic tongue is a system that usually consists of an array of non-specific chemical sensors combined with appropriate data acquisition systems and chemometric tools. During sample assessment the ET sensor array produces an unresolved analytical signal, which is correlated with the chemical composition of the sample. This relies on the proper choice of the sensor array, comprising sensors with pronounced sensitivity towards the substances and properties of interest. The resulting signal can be processed by various multivariate data analysis techniques to extract quantitative and qualitative information about the sample. Typically classic chemometric methods such as PCA (principal component analysis), PLS, SIMCA (soft independent modelling of class analogy), PLS–DA (partial least squares–discriminant analysis) have been widely employed for this task, though ANN (artificial neural network) implementations have also been reported [14,15]. The ET systems based on different sensing principles (potentiometry, voltammetry, etc.) have proved to be promising analytical tools for wine analysis, however, there is definitely a strong need for further development and acceptance by wine producers [16,17].

Most of the papers devoted to the application of the ET to wine analysis have dealt with classification tasks. Various types of the ETs capable of classifying wine samples according to grape variety [18,19], denomination of origin [20], ageing type [21,22], sugar content [23], etc. are described in the literature. In recent years there has been a growing number of research papers devoted to the numerical prediction of different wine quality parameters, both chemical and sensory. Total polyphenols, total and volatile acidity, pH, individual taste descriptors, etc. can be predicted in wines from the ET response [19,23–26]. Some of these results have become almost trivial, although there are a number of problems that still persist.

PLS regression is the most widely used method to obtain calibration models for numerical predictions of various quality parameters. The predictive ability of PLS regression models should be properly evaluated before any kind of real life application of these PLS models can be considered. The results available in the literature indicate that researchers usually do not pay serious attention to a realistic estimation of the predictive ability of PLS models. In the vast majority of papers, numerical parameters for the regressions (such as RMSEP, offset, R^2, slope) are reported for the validation procedure based on a full cross-validation or a single random split test set validation. However, these parameters often do not suggest a realistic estimate of the further predictive power of the model, since the same objects (samples) are used for the development and validation of the model. These issues are widely described in the chemometric literature [27–31]. Cross-validation is widely known to produce over-optimistic results and can only serve as a rough estimation of model performance. Test set validation is a more preferable option, but it requires a large number of samples for training, optimization and evaluation of predictive ability of a model. A large number of different samples is rarely available for the ET in the wine research, because all of the samples should be evaluated with various reference techniques (instrumental, sensory panel etc.), and this can be expensive and at time impractical. We also believe that the single random split test set, which is also widely employed in the ET field, is not the best choice, since one random choice of samples for validation may not be very representative, and thus can lead to both over-optimistic or over-pessimistic conclusions about model performance. This is typical not only for ET applications in wine analysis but for many other areas as well.

A possible approach to obtain a more realistic forecast of the performance of regression models for an ET can be a k-fold random split test set. This approach will not operate with only one sample excluded from the modelling for prediction, and will not estimate the RMSEP with only one split. The initial sample set of k samples is split into two parts: the first m samples are used for training (calibration), and the second n samples are utilized as an independent test set for the assessment of the predictive power of the model [28]. Obviously, in the ideal situation all possible C_n^k combinations have to be tested, however this could require significant computational time. This validation approach is widely suggested in chemometrics, but is far from being a common practice with ET data processing.

The objective of this work is to compare three methods for assessment of the predictive power of PLS1 regression models obtained for quality parameters, both chemical and sensory, in red and white wines on the basis of the data obtained using a multisensor system. Full cross-validation, single random split test set and k-fold random split test set were studied. Blaufränkisch wines from Slovakia were chosen for a case study of the correlation between sensory descriptors and the ET response. White wines from New Zealand, consisting of Sauvignon Blanc, Chardonnay and Pinot Gris, were used to determine the correlation between the ET and traditional chemical analyses.

2. Experimental

2.1. White wine samples

The first part of the study is devoted to an assessment of the correlation between the ET response in white wine samples and various chemical parameters. This was undertaken for a sample set containing 36 different white wines commercially produced in New Zealand. Three wine varieties were involved in the study: Pinot Gris (4 samples), Chardonnay (16 samples) and Sauvignon Blanc (16 samples). The wines were produced in vintages between 2002 and 2008, and were taken from the University of Auckland wine library. The wines originated from several regions within New Zealand, including Marlborough, the Hawke's Bay and Nelson (Table 1).

2.2. Red wine samples

The second part of the study deals with the correlation between ET analysis results and sensory panel scores. 27 samples of red Slovak Blaufränkisch wines were selected for this research. The samples were acquired from two sources. The first group of wines were purchased from a local store in Nitra, Slovakia, and the second group of boutique wines were provided by the UKSUP (Central and Testing Institute in Agriculture), Bratislava, Slovakia. Two bottles of each sample were obtained – one for sensory panel analysis and one for ET measurements. The geographical origin of the samples covers the west vineyard region (16 different locations), middle (2 locations) and eastern parts (6 locations) of Slovakia. All wine samples were transferred to nonwhite glass bottles and stored under the same conditions before sensory and ET analysis.

2.3. Sample preparation for ET measurements

To ensure the consistency of wine chemical composition throughout the analyses and procedures, the following scheme for sample preparation of white wines was used: once the bottle was opened, 20 mL of wine was taken for reference SO_2 analysis. The rest

Table 1
The origin of the white wine samples.

Origin	Vintage (no. of samples)		
	Chardonnay	Sauvignon Blanc	Pinot Gris
Hawke's Bay	2002(1), 2004(3), 2005(2), 2008(1)	2005(2), 2007(1)	
Marlborough	2002(1), 2004(1), 2005(2), 2006(1)	2003(1), 2004(2), 2005(4), 2006(2), 2007(1), 2008(1)	2004(1), 2007(1)
Nelson	2006(1)	2005(2)	
Gisborne	2004(1)		
Waipara valley	2004(1)		
Waiheke Island	2007(1)		
West Melton			2006(1)
South Canterbury			2006(1)

of the bottle was poured into five 50 mL plastic containers for ET measurements, one plastic container for the FOSS WineScan analyzer and two small ampoules for polyphenolic analysis by HPLC with diode array detector, as described previously [32]. All of these containers were frozen in liquid nitrogen and afterwards stored at −80 °C. Thawing and thermostating of the sample at +22 °C was performed immediately before the ET measurement for each sample. Obviously there is no need for these extra steps in routine ET applications, but only for research purposes, when replicates of the same wine are analyzed on different days.

Red wine samples were prepared for ET measurements in a similar way. Right after opening the bottle its contents was poured into seven 50 mL plastic containers and were frozen at −20 °C to ensure invariability of chemical composition. Thawing and thermostating at +22 °C was undertaken just before the ET measurement.

The white and red wines were frozen under different conditions as the two experimental sessions were carried out in two different years and at two different locations. The first session was held in New Zealand in 2008, with white wine samples and standard chemical analysis (for SO_2, acidity, phenolics); the second experiment was undertaken separately in Slovakia and Russia in 2009 and 2010, with red wines and a sensory panel.

2.4. Electronic tongue measurements

Two different sensor arrays were employed in this study – one for white and one for red wines. All sensors, except the pH-glass electrode, were developed and produced in the Laboratory of Chemical Sensors of St. Petersburg University. Further details on the preparation procedures and materials can be found elsewhere [33]. Electrochemical measurements were carried out in the following galvanic cell:

$$\mathrm{Cu}\,|\mathrm{Ag}|\,\underline{AgCl},\ \mathrm{KCl_{sat}}|\ \text{sample solution}|\ \text{membrane}|\ \text{inner contact}|\,\mathrm{Cu}.$$

Electromotive force (sensor potential) values were measured with 0.1 mV precision against a standard reference electrode using a custom made 32-channel digital high impedance voltmeter connected to a PC for data acquisition. A glass pH electrode was used to monitor and control the pH value of sample solutions.

The sensor array for the white wines consisted of 25 potentiometric cross-sensitive sensors, 14 of which were PVC-plasticized anion-sensitive, 6 PVC-plasticized cation-sensitive, 5 chalcogenide glass sensors with various types of red/ox sensitivity and one standard pH glass electrode. The sensor array used for the red wines involved 27 potentiometric chemical sensors: 10 PVC-plasticized anion-sensitive, 12 PVC-plasticized cation-sensitive, 4 chalcogenide glasses and one pH glass sensor. At least 5 replicate ET measurements were taken for each wine with physically different samples from the same bottle. The samples were diluted with Milli-Q water in the ratio 30 mL wine/70 mL water before the measurement. The measurement time in the sample was 3 min and after that the sensor array was washed several times with distilled water to return the sensor readings back to their initial states. Replicates of different wine samples were measured in a random order. Data processing was performed with sensor responses averaged over 5 measurements.

2.5. Reference chemical analysis of white wines

All 36 white wines were analyzed by standard chemical techniques. Reference SO_2 analysis was performed with the standard aspiration method [34], which yields free, bound and total sulfur dioxide content. A FOSS WineScan^{TM} FT120 operated at the Pernod Ricard winery in Glen Innes, Auckland, was used to determine the total acidity, volatile acidity, ethanol content, pH and concentration of reducing sugars. An HPLC with diode array detector was employed for phenolic analysis yielding concentrations of catechin, epicatechin, gallic acid, caffeic and coumaric acid, as described previously [32]. In brief, about 2 mL of wine was filtered through a 0.45-μm cellulose filter (Minisart RC-4), of which 20 μL was injected into a Phenomenex Luna C18 column (4.6 × 250 mm, 5 μm particle size) (Torrence, CA) on an Agilent 1100 series instrument (Waldbronn, Germany). A ternary solvent was run at a flow rate of 0.8 mL/min employing (A) water, (B) 5% aqueous acetic acid, and (C) acetonitrile. The initial gradient composition was 45%A and 55% B, and over the course of 20 min the gradient was changed linearly to 100% B. From 20 to 50 min the gradient moved to 90%B and 10%C, and from there to 55% B and 45%C by 70 min. While 55% B was maintained constant over the next 20 min, the gradient shifted from 45% C to 45% A, giving a total run time of 90 min. The phenolics were identified using a combination of commercial standards and the UV–vis spectra of peaks in comparison with published procedures, as described previously [32].

2.6. Sensory evaluation of red wines

Red wine samples were thermostated in bottles at room temperature and were opened immediately before the sensory analysis sessions. Three experts with ISO/IEC 17024 certificates for the analysis of wine and 20 years of experience in the local wine

industry were recruited. Seven taste attributes, including astringent, acid, spicy, plum, berries, fullness and off-taste were developed and defined in collaborative dry run sessions. A description of the sensory attributes is as follows: astringent – the degree of astringent/bitter taste/flavour; acid – the degree of acid/sour taste/flavour; spicy – the degree of spicy/hot (spice, pepper) taste/flavour; plum – the degree of plum and associated (dry plums, plum jam tones) taste/flavour; berries – the degree of berries and garden fruit; harmony/fullness – the ratio of all taste/flavour components percieved by the human receptor; off-taste – the degree of all other taste/flavour attributes. Sensory analysis of the samples was performed in six individual sessions at the Sensory Laboratory of the Slovak Agricultural University (SAU) in Nitra, Slovakia. The samples were evaluated in standard glasses for the sensory analysis of wine according to ISO 3591:1977, and were served in a randomized and balanced order. Judges used a 9-point intensity scale for each attribute where 1 means "missing" and 9 is "extremely strong". The judges used water and slices of white bread between the evaluation of samples to refresh taste buds and eliminate the fatigue effect. The data obtained by sensory evaluation were averaged and processed in electronic data sheets.

2.7. Data processing and software

A study of the correlations between ET data and chemical analysis data for the white wines, and between ET data and sensory data for the red wines, was undertaken by principal component analysis (PCA), canonical correlation analysis (CCA) and partial least squares (PLS) techniques. CCA is intended for the description of the correlation between two data sets obtained by various analytical methods for the same sample set [35]. In our case it was not possible to run CCA on the initial data sets due to their size (too many variables compared to the number of samples). In this situation PCA can be used for effective data compression without significant information loss and after that CCA can be run on the resulting PC scores for both data sets. This approach was implemented to relate ET data to the sensory data for the red wines, and with chemical analyses on the white wines.

All replicated ET measurements were averaged for data processing, thus the initial data set for the white wines was 25 (sensors) × 36 (samples) matrix, and for the red wines, 27 (sensors) × 27 (samples) matrix.

The relationship between sensory and ET data was studied by running PLS2 regression on the ET data set for red wines as independent variables to predict sensory descriptors as dependent variables. Further on, separate PLS1 models were built for each correlated sensory descriptor.

The relationship between ET response and chemical analysis parameters for white wines was studied by PLS1 regression. PCA and PLS models were computed with The Unscrambler® 9.7 (CAMO Software AS, Norway). CCA was performed using R 2.10.1 statistical computation package [36].

Estimation of the predictive ability of the PLS models was undertaken using three different approaches: full cross-validation; single random split test set and k-fold random split test set. The last procedure was performed by randomly splitting the samples into calibration (around 2/3rd of the samples) and test set (around 1/3rd of the samples). This random split was repeated 20 times and for each run a root mean square error of prediction (RMSEP) was calculated:

$$\mathrm{RMSEP} = \sqrt{\frac{\sum_{i=1}^{n}(y_{i,pred} - y_{i,ref})^2}{n}} \quad (1)$$

where n is a number of samples in the test set, $y_{i,pred}$ is the value predicted by model, $y_{i,ref}$ is the reference value. After that RMSEP values obtained in 20 different splits were averaged and this final averaged RMSEP was a much more realistic assessment of the predictive ability of the models, compared to a simple reporting of regression parameters either for cross-validated models or for validations based on a test set with a single random split of the samples. This was due to the fact that this RMSEP was obtained for a number of completely independent test sets. Obviously the number of samples that is kept out from the model training can be lower than 1/3rd. While making these random splits it is important to make sure that every sample was employed at least once in a test set.

3. Results and discussions

3.1. Relation between ET and instrumental chemical analysis datasets. Case study of New Zealand white wines

To examine correlations between ET and instrumental chemical analysis results, CCA was initially employed in the following way. For the given sample set of 36 wines we had 26 ET sensors and 13 instrumental chemical parameters. Thus CCA was run not on the original variables, as they were too numerous, but on the PCs extracted with PCA from the ET and instrumental chemical parameter matrices. This approach is usually used with large spectroscopic data sets [37]. PCA was run separately on ET and instrumental chemical data for the same 36 white wine samples. Scores of the 6 resulting PCs for ET data, accounting for 94% of total dispersion, and scores of the 8 resulting PCs for CA data, accounting for 93% of the total dispersion, were chosen and CCA on the corresponding PC scores was computed. Six pairs of canonical variates were extracted with squared canonical correlation coefficients of 0.93, 0.80, 0.69, 0.58, 0.38 and 0.10, respectively. The first two canonical roots imply that a reasonably good description of instrumental chemical analysis results is possible using the ET data.

Similarity maps defined by canonical variates 1 and 2 for ET and instrumental chemical analysis are shown in Fig. 1. In this plot samples 1–16 are Chardonnay, 17–20 are Pinot Gris, and 21–36 are Sauvignon Blanc wines. A reasonable match of both maps can be observed. There is some separation between Chardonnay and Sauvignon Blanc along the CV1 axis, however no clear clustering according to grape variety was observed for both datasets and significant overlapping between different wines is shown. This is quite understandable taking into account the comparatively small number of determined chemical parameters and the complex wine chemistry involved. However on the PCA score plots for ET data this overlapping was expressed even more and no separation along the PCs was clearly visualized.

3.1.1. Determination of SO_2 in white wines

To explore the possibilities of numerical prediction of instrumental chemical parameters from the ET response, PLS1 regression was used. Instrumental chemical parameters were considered as dependent variables and were predicted with the set of independent variables – the ET sensor responses.

The first parameter of interest was sulfur dioxide (SO_2) concentration. SO_2 has a long history in winemaking owing to its antimicrobial and antioxidative properties [38]. SO_2 occurs in all wines as a by-product of yeast metabolism [39], but it is also added at various stages in winemaking, from grape crushing to wine bottling, in various forms (liquefied gas, solutions of salts potassium metabisulfite ($K_2S_2O_5$), sodium metabisulfite (not a legal additive for wine production in some countries)). The bisulfite ion (HSO_3^-) is the dominant form at wine pH, and combines with a wide range of substances such as acetaldehyde and anthocyanins to produce 'bound forms'. Alongside the beneficial effects of SO_2 addition to wines, there is some concern to limit the amount used because

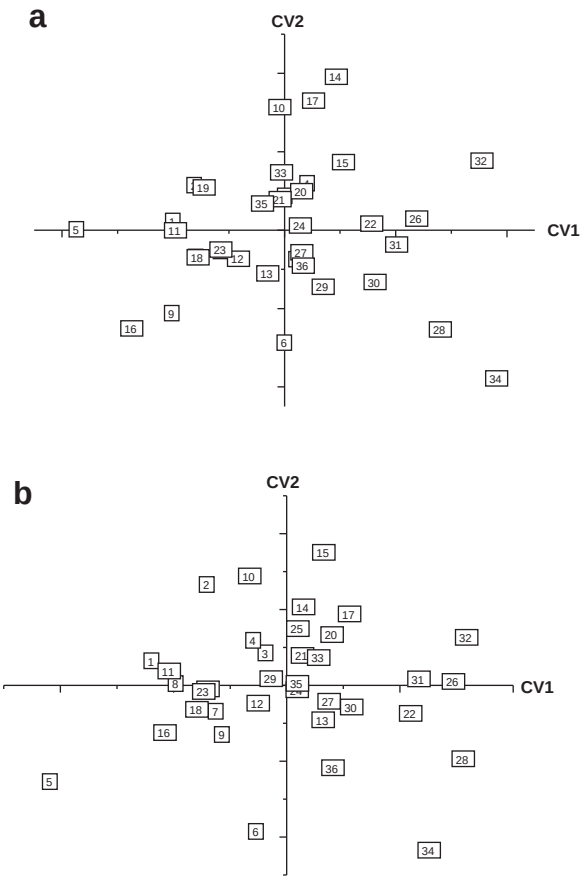

Fig. 1. Canonical correlation analysis similarity map defined by canonical variates 1 and 2, for (a) instrumental chemical analysis, and (b) ET measurements of 36 white wine samples.

of associated health issues such as asthma and allergic reactions [40–42]. Thus, SO_2 levels in wines should be accurately controlled to ensure sufficient SO_2 is present for its beneficial role in winemaking, but no more than is necessary.

There are a number of standard methods for SO_2 determination in wine such as the Ripper method, Paul method and aspiration method. Although the accuracy of these methods is rather high, they still suffer from a number of drawbacks: complexity, time consumption and lack of automatization possibilities.

As a first step a calibration of the ET against reference data (obtained using the standard aspiration technique for SO_2) was performed by PLS1 regression for the whole data set (36 white wines). The observed correlation between ET response and reference free SO_2 content was rather poor (R^2 around 0.6 in full cross-validation). A probable reason for this was the significantly different wine chemistry for the various grape varieties. This makes matrix effects in the sample dominating, and thus hinders SO_2 determination by the sensors of the array in the frame of one global PLS model.

Thus separate PLS1 models for Sauvignon Blanc (16 samples) and Chardonnay (16 samples) wines were computed using the aspiration technique data on free and total SO_2 as a reference. No calculations were undertaken for Pinot Gris wines since there were only 4 samples of this variety available. Three different validation modes for these models were tested for comparison: full cross-validation (leave one out validation), test set validation with a single random split (10 samples for calibration and 6 samples for validation) and the k-fold random split test set validation described in Section 2. The number of splits was 20, as we found in our preliminary calculations that after 20 splits the averaged RMSEP value did not change significantly after adding results from new splits. The validation parameters of the models for measured vs predicted plot are summarized in Table 2.

The validation parameters confirm the very good correlation of ET response with SO_2 content. The comparison between RMSEP values obtained in different validation modes highlights a well-known problem with cross-validated models – the estimation of the prediction error is over-optimistic. RMSEP values obtained with k-fold random split are always higher than those obtained in cross-validation and in test set validation with 6 randomly chosen samples. In case of total SO_2 prediction in Sauvignon Blanc wines, RMSEP yielded by k-fold random split was almost 3 times higher than the value from the full cross-validation. This fact implies the relevance of using more reliable validation methods than a test set with a single random split or cross-validation, as are usually applied for PLS models in ET research.

3.1.2. Determination of pH, ethanol and acidity in white wines

The parameters determined by the FOSS WineScan instrument, including total acidity, volatile acidity, pH, reducing sugars and ethanol, were also studied for correlations with the ET response. PLS1 modelling with three different validation modes was used for all of the parameters. ET data for all 36 wine samples were employed. No significant correlation was found between ET data and reducing sugars as these substances are not ionized at normal wine pH values. Further, there were no correlations observed for volatile acidity, probably because their concentrations (as determined by FOSS Wine Scan instrument) were very low (0.21–0.68 mg/L), and in the complex wine matrix determination of such low contents will be significally hindered by other anionic spices present at a higher concentration. The wine acid content is a very important wine parameter for the sensory impact of a wine, and the sugar/acid balance is one of the major wine taste parameters that winemakers pay great attention to in their winemaking. For this purpose both wine pH and wine total acidity (typically 6–9 g/L) are measured through chemical analysis procedures. The resulting validation parameters are shown in Table 3.

Total acidity and pH can be determined with reasonable accuracy using the ET system. For pH prediction only 3 variables (sensors of the array) were used, namely one chalcogenide glass sensor, one cation-sensitive and one anion-sensitive sensor. No standard pH glass sensor was employed in this PLS1 model. Each of these sensors is pH-sensitive to some extent, thus their combination allows for rather accurate pH determination without a pH glass sensor. Total acidity and pH for this particular data set were correlated with R^2 0.61, however in general there is no direct connection between total acidity and pH in wine. An RMSEP comparison for different validation modes shows that PLS models for total acidity and ethanol as validated by full cross-validation and test set with a single random split tends to produce over-optimistic estimation of prediction. Another important observation is that cross-validated models are more complex in terms of LV number as they are too greatly tuned for the fitting of calibration data.

A poor connection between the ET data and ethanol content is not surprising since C_2H_5OH is not expected to be ionized at normal wine pH. There is a report by Lvova et al. [43] on the application of potentiometric ET for the detection of alcohols in beverages, but the sensor array in this study was different, and, more importantly, the concentration range where alcohol sensitivity was observed by authors of [43] was much wider: 10^{-5} to 1 mol/L, instead of the 2.5–3 mol/L ethanol concentration range that we have in this study. This narrow range hinders precise determination of ethanol in white wines using the suggested ET system due to a lack of sensitivity.

Table 2
Parameters of ET performance for prediction of free and total SO_2 obtained by three different validation modes.

Validation mode	Slope	Offset	RMSEP, mg/L	R^2	#LV
Sauvignon Blanc, free SO_2 (2–24 mg/L)					
Full cross-validation	0.75	3.8	3	0.81	2
Test set validation with a random split, 6 samples	0.70	4.4	2	0.82	1
Test set with 5 samples 20-fold random split	–	–	4	–	–
Sauvignon Blanc, total SO_2 (35–126 mg/L)					
Full cross-validation	0.73	24.7	4	0.70	2
Test set validation with a random split, 6 samples	0.78	12.2	10	0.64	2
Test set with 5 samples 20-fold random split	–	–	14	–	
Chardonnay, free SO2 (6–26 mg/L)					
Full cross-validation	0.86	2.1	2	0.89	2
Test set validation with a random split, 6 samples	0.71	5.7	3	0.79	2
Test set with 5 samples 20-fold random split	–	–	4	–	
Chardonnay, total SO_2 (49–136 mg/L)					
Full cross-validation	0.77	19.8	9	0.84	3
Test set validation with a random split, 6 samples	0.78	16.4	10	0.84	2
Test set with 5 samples 20-fold random split	–	–	25	–	

Table 3
Parameters of ET performance for prediction of total acidity, pH and ethanol obtained by three different validation modes.

Parameter (range)	Slope	Offset	RMSEP, mg/L	R^2	#LV
Total acidity (5.03–9.09 g/L)					
Full cross-validation	0.90	0.69	0.31	0.86	4
Test set validation with a random split, 16 samples	0.84	1.28	0.45	0.80	3
Test set with 12 samples 20-fold random split	–	–	0.64	–	–
pH (3.08–3.63)					
Full cross-validation	0.95	0.17	0.04	0.95	2
Test set validation with a random split, 16 samples	0.89	0.36	0.04	0.97	1
Test set with 12 samples 20-fold random split	–	–	0.04	–	–
Ethanol (11.41–14.55 vol%)					
Full cross-validation	0.71	3.82	0.28	0.70	5
Test set validation with a random split, 16 samples	0.55	5.98	0.46	0.61	2
Test set with 12 samples 20-fold random split	–	–	0.61	–	–

3.1.3. Determination of phenolic compounds in white wines

The HPLC data on the concentration of phenolic compounds in wines were used to study the ET capabilities for determination of this important class of wine ingredients. Phenolic compounds are widely known to contribute to the taste and colour of wines [44], and their quantification by means of rapid and inexpensive techniques is of considerable interest in wine science [45]. The concentrations of caffeic acid, catechin, coumaric acid, epicatechin and gallic acid were quantified by HPLC for all of the 36 white wine samples. When using the whole HPLC wine analysis data set for ET calibration the corresponding R^2 was lower than 0.7, probably due to a strong matrix effect. For further experiments we decided to build separate models for the phenolic contents in the two wine varieties: Chardonnay and Sauvignon Blanc. We did not use full cross-validation option here taking into account the above discussion, thus only two validation methods were employed: single split test set and k-fold random split. Data on the RMSEP values of the models are presented in Table 4.

There was no valuable correlation with the ET data observed for the epicatechin content for both Sauvignon Blanc and Chardonnay. The correlation with catechin was somewhat higher but was also rather poor, thus they are not reported in Table 4. This fact can be explained by dissociation degree of these polyphenolic compounds. Since the potentiometric ET platform allows for direct determination only of ionized substances (however, in some cases an indirect correlation with non-ionic spices can also be observed), it requires a significant amount of the ionic forms of substances to be present in the sample. When comparing the pK_a values of the studied polyphenols, it can be seen that epicatechin and catechin are very weak acids compared to caffeic, coumaric and gallic acids (pK_{a_1} values around 8.2 and 4.3, respectively) [46,47].

3.2. Relation between ET response and sensory panel. Case study of Slovak red wines

The set of Blaufränkisch red wines, produced from different regions of Slovakia, was analyzed by an ET sensor array and by a skilled expert sensory panel. The colour of Blaufränkisch wines varies from red ruby tones in young wines to a purple tone in old wines. Major flavour trends with these wines are moderate

Table 4
Parameters of ET performance for prediction of phenolics obtained by two different validation modes.

Polyphenol (range, mg/L)	RMSEP, mg/L by a single split test set validation	RMSEP, mg/L by test set with 5 samples 20-fold random split
Sauvignon Blanc		
Caffeic acid (1.6–4.9)	0.43	0.69
Coumaric acid (0.8–4.9)	0.14	0.65
Gallic acid (0.7–1.3)	0.02	0.12
Chardonnay		
Caffeic acid (0.8–4.2)	0.20	0.76
Coumaric acid (0.2–2.5)	0.37	0.49
Gallic acid (0.4–4.7)	0.31	1.42

Table 5
Parameters of ET performance for prediction of sensory descriptors obtained by three different validation modes.

Validation mode	Slope	Offset	RMSEP, points	R^2	#LV
Acids (1–5.7)					
Full cross-validation	0.88	0.4	0.4	0.86	5
Test set with 11 samples, single random split	0.99	0.2	0.6	0.79	3
Test set with 9 samples 20-fold random split	–	–	0.6	–	–
Astringent (1.3–6.7)					
Full cross-validation	0.72	0.8	0.4	0.71	4
Test set with 11 samples, single random split	0.87	0.5	0.5	0.82	2
Test set with 9 samples 20-fold random split	–	–	0.8	–	–
Berries (1.7–6.7)					
Full cross-validation	0.81	0.7	0.5	0.82	3
Test set with 11 samples, single random split	0.91	0.6	0.6	0.83	3
Test set with 9 samples 20-fold random split	–	–	0.7	–	–
Spicy (1–5.7)					
Full cross-validation	0.76	0.7	0.4	0.75	2
Test set with 11 samples, single random split	0.84	0.5	0.3	0.75	2
Test set with 9 samples 20-fold random split	–	–	0.6	–	–

tannins and harmonous acids; plum marmalade and raspberry tones are often recognized. Some mature Blaufränkisch wines can also have the notes of blackberry, cinnamon, bitter chocolate, and walnuts. Young wines are more fruity. Seven taste attributes, namely acid, spicy, plum, berries, fullness and off-taste were assessed using a 9-point scale (1-missing, 9-extremely strong).

To explore the correlations between these data sets, PLS2 regression was computed. Independent variables – the ET sensor responses, were used to predict a set of dependent variables – the sensory descriptors. A correlation loadings plot for the first two latent variables [48] was used to assess the correlation of the ET with particular taste attributes (Fig. 2).

The inner ellipse in Fig. 2 indicates 50% of the explained variance and the outer ellipse is the unit-circle indicating 100% of the explained variance (or the absolute values of the correlation coefficient of 0.7 and 1 respectively). Thus, the sensory parameters between the two ellipses can be considered as correlated with the ET response, and those inside the inner ellipse are poorly connected with the ET data. Five latent variables were extracted, containing 86% and 62% of the total variance in ET the data and sensory panel data, respectively.

It can be seen that 4 descriptors: acids, astringent, berries and spicy can be estimated from the ET data, while parameters such as plum, harmony and off-taste are not well described with the potentiometric sensor array. A possible explanation for this fact is the complex chemical nature of the descriptors, particularly, harmony and off-taste. Obviously, there is no clearly defined chemical substance or group of substances in the red wine that can be responsible for its "harmonic" taste. This parameter is too general and is more connected with individual assessor training experience than with a certain chemical substance or composition. A similar reasoning can be put forward for the "off-taste" descriptor. It is "the degree of all other taste/flavour attributes", and thus can be related to any chemical substances in the wine that are not described by the rest of identified sensory attributes. This generality of chemical background likely leads to poor prediction of such attributes from the ET response.

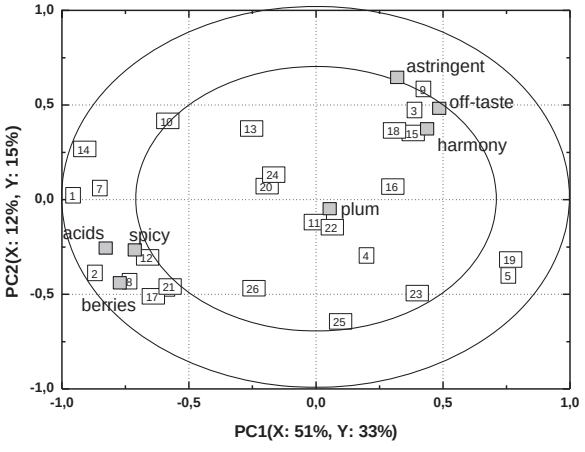

Fig. 2. PLS2 correlation loadings plot for latent variables 1 and 2. The ET sensors are denoted as numbers in squares, taste descriptors are denoted as grey squares with the corresponding attributes written nearby. The amount of total variance captured by each PC for ET data (X) and sensory data (Y) is shown in the curly brackets.

Separate PLS1 models for prediction of 4 correlated sensory descriptors in red wines were computed. In Table 5 parameters of validation of these PLS1 models are shown.

It was found that the ET permits good prediction of acid, astringent, berries and spicy attributes (RMSEP is 0.5–0.8 points on the sensory scale). Standard deviation values for averaged assessor scores were in the range of 1–1.6 points for all of the descriptors. The RMSEP values obtained in the different modes were different and the k-fold random split approach tended to give the highest RMSEP, as in the case of the correlation between ET response and chemical analysis data for the white wines. Test set validation with a single random split of samples into calibration and validation subsets in some cases provided higher slope and R^2 values than for a full cross-validation. This can be attributed to the random choice of samples for validation with significant probability of chance correlation, and implies the need to use more reliable validation methods for the estimation of the predictive ability of the models to avoid over-optimistic conclusions about the preformance of an artificial sensory system.

4. Conclusions

The results of the present study confirm that an "electronic tongue" sensor system based on a potentiometric platform, supported by classic chemometric techniques, is a promising tool for wine analysis for both chemical and sensory characterisation. While not competing in precision with standard chemical analysis methods, the ET offers the advantage of rapid and simple measurements, and can be adopted in the future as a quick tool for quality monitoring in the wine industry. Sensory scores prediction by the ET can also find wide application in wine analysis. However,

proper care should always be given to the chemistry behind the corresponding descriptors when developing an ET for a particular application. Realistic assessment of the predictive ability must be an essential part of the development of an artificial sensory system. The k-fold random split procedure employed in this paper can be a more realistic alternative for the full cross-validation and single split test set validations that are widely used in the literature with sensory systems. The k-fold random split test set validation provides a reliable estimation of predictive ability of multivariate regression models. It does not require significant computational efforts and can be used on relatively small sample sets, as are usually available in wine research.

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