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Detection of copper, lead, cadmium and iron in wine using electronic tongue sensor system

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ABSTRACT

An array of 10 potentiometric chemical sensors has been applied to the detection of total Fe, Cu, Pb and Cd content in digested wine. As digestion of organic matter of wine is necessary prior to the trace metal detection using potentiometric sensors, sample preparation procedures have been optimized. Different variants of wet and microwave digestion and dry ashing, 14 conditions in total, have been tested. Decomposition of organic matter was assessed using Fourier transform mid-infrared spectroscopy and total phenolic content. Dry ashing was found to be the most effective method of wine digestion. Measurements with sensors in individual solutions of Fe(III), Cu(II), Pb(II) and Cd(II) prepared on different backgrounds have shown that their detection limits were below typical concentration levels of these metals in wines and, in the case of Cu, Pb and Cd below maximum allowed concentrations. Detection of Fe in digested wine samples was possible using discrete iron-sensitive sensors with chalcogenide glass membranes with RMSEP of 0.05 mmol L^{-1} in the concentration range from 0.0786 to 0.472 mmol L^{-1}. Low concentration levels of Cu, Pb and Cd in wine and cross-sensitivity of respective sensors resulted in the non-linearity of their responses, requiring back-propagation neural network for the calibration. Calibration models have been calculated using measurements in the model mixed solutions containing all three metals and a set of digested wine sample. RMSEP values for Cu, Pb and Cd were 3.9, 39 and 1.2 μmol L^{-1} in model solutions and 2, 150 and 1 μmol L^{-1} in digested wine samples.

1. Introduction

Trace metals are commonly present in wine usually coming from the two main sources: environmental, i.e. soil on which grapevine is grown, contamination originating from cars, factories, etc., and anthropogenic, including use of fertilizers and pesticides, and oenological practices (machinery, piping, use of fining agents, additives, etc.) [1,2]. Content of trace metals in wine has to be controlled as due to their adverse effect on human health and wine quality.

Iron and copper are the two most widely studied metal ions in relation to oxidative spoilage because they have the capacity to activate molecular oxygen as well as catalyze the degradation of hydrogen peroxide to the hydroxyl radical [3]. Copper originating from either vineyard sprays or copper-based equipment, is mostly precipitated by yeast cells during and after fermentation and copper concentration of freshly fermented wine is generally quite low, less than 0.1 mg L^{-1}. The main source of copper in finished wine is a result of oenological practice of adding copper(II) sulfate for the removal of sulfuric off-odours. The added copper(II) precipitates as copper sulfide, which can be separated by settling mg L^{-1} or filtration. As copper is usually added in excess to the sulfide present in wine to ensure complete removal of the latter, residual copper(II) concentrations in the wine can be high [4–6]. High residual copper(II) has been linked to an enhanced rate of oxidation, which ultimately results in the browning of the wine, especially white wine. Copper(II) can also contribute to the formation of hazes (copper casse), which often appear only after the wine is bottled [7–9]. Maintaining copper concentration in finished wine below 0.3–0.5 mg L^{-1} is recommended for minimization of the spoilage occurrence [10]. It is important to note that at higher levels copper(II) is also toxic and therefore, maximum acceptable concentration of copper(II) in wine, which is considered safe for human consumption, is established at 1 mg L^{-1} [11]. Typical copper levels reported in wine vary between 0.02 and 3 mg L^{-1} [1,5,6].

Another metal contributing to wine spoilage is iron. Iron capacity to form insoluble iron(III) phosphate and protein complexes leads to the haze formation commonly known as iron casse. Another iron effect is due to its involvement in oxidative processes, often leading to enhanced coloration or browning of wine [3,12]. Iron is not toxic and, thus, no maximum acceptable concentration is established for it. Recommendations about maximum total iron

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concentration permitting to avoid wine spoilage are not consensual and typically vary between 7 and 15 mg L^{-1} [1,12].

Metals such as Cd, and Pb, have no nutritional value or physiologic role in the human organism, but recognized to be highly toxic and, thus, their concentration in wine should be kept at as low as possible. Maximum allowed concentrations established for lead and cadmium in wine are 150 and 10 μg L^{-1}, respectively [11]. Control of lead concentration in wine is particularly important as lead contamination from the environmental sources and equipment such as lead plumbing in wineries is quite widespread [13]. Consequently, reported lead levels in wine vary widely: concentrations between 1 and 1125 μg L^{-1} have been reported [1,5,6]. This means that lead content frequently exceeds safe amounts. Wine contamination by cadmium is less problematic with reported levels in wine varying between 0.1 and 8.1 μg L^{-1} [1,5,6].

The most commonly used techniques of the detection of the total content of trace metals in wine are Atomic Absorption Spectroscopy, Electrothermal Atomic Spectrometry, Inductively Coupled Plasma Mass Spectrometry and Optical Emission Spectrometry [2,14,15]. Though these methods afford high accuracy and selectivity and very low detection limits, instrumentation and its exploitation costs are quite high and qualified personnel is required to use them. Moreover, and in order to reach the low concentration levels required, these techniques might be accompanied by labor-intensive sample treatment stages, liquid-liquid, or solid phase extractions. Electrochemical sensors represent an attractive alternative as a simple, rapid, easily automated and cost effective method for the trace metal determination. Electrochemical sensors respond to the free (potentiometric) or labile (voltammetric) metal ion concentration. As wine is highly complexing medium, trace metals will be mostly bound to organic ligands primarily with polyphenols and proteins, and, therefore, sensors cannot be applied directly to the determination of the total trace metals.

Electrochemical techniques have been applied to the trace metal speciation in wine, in particular, concentrations of free copper and lead were measured using direct potentiometry with ion-selective electrodes [16–18], concentrations of labile forms of lead and copper were measured using square wave cathodic voltammetry on multimode mercury electrode [17–19] and stripping anodic voltammetry on carbon glass electrode [10,20]. Voltammetric techniques are more selective compared to direct potentiometry, however, techniques applied to the metal detection in wine involve use of mercury electrodes or mercury containing stripping solutions, which is undesirable. Insufficient selectivity of potentiometric chemical sensors in multicomponent media is well known and can be successfully counteracted by using arrays of sensors with chemometric data treatment instead of discrete sensors [21,22]. Several applications of sensor arrays or electronic tongues based on the potentiometric chemical sensors for simultaneous quantification of transition metals in model solutions have been reported [23–28]. In particular, simultaneous detection of Pb^{2+}, Cr(VI), Cu^{2+}, Cd^{2+} in the incineration plant smoke after their absorption by the acidic solutions using flow-injection set-up [23], detection of Cu^{2+}, Zn^{2+}, Mn(II), Fe(III), Ca^{2+} and Mg^{2+} in the model groundwater [24], determination of Ag^{+}, Cu^{2+} and Pb^{2+} in the model solutions using microsensors [25], detection of Cd^{2+}, Cu^{2+}, Pb^{2+}, Zn^{2+} and Ca^{2+} in the model solutions [26,28] and soils extracts [27] using flow injection and sequential injection systems. Artificial neural networks were used for the calibration of the sensor arrays.

Free or labile forms of trace metal are bioavailable and act as catalysts in oxidation processes. Therefore, trace metal speciation in wine rather than total concentration is more relevant for assessing both their toxicity and potential for spoilage. However, currently there is not enough data available about safe or desirable levels of free and labile metal forms and all recommended and maximum allowed concentrations of metals in wines are expressed in terms of their total concentrations. Thus, in the present work we aimed at the application of an array of potentiometric chemical sensors for detection of total trace metal content in wine. Obviously, in this case sample preparation i.e. digestion of wine organic matter is required prior to measurements. Introduction of sample preparation step increases analysis time and complicates procedures diminishing advantages of using electronic tongue for this purpose. On the other hand, wine digestion is commonly used before trace metal analysis using spectral techniques [29–33]. Inaccuracy of the results obtained using simple wine dilution instead of digestion has been highlighted in the literature [29].

The present study reports on the application of the electronic tongue based on potentiometric chemical sensors to quantitative detection of metals such as iron, copper, lead and cadmium in digested and whole wine and optimization of wine digestion procedure.

2. Materials and methods

2.1. Reagents

Nitric acid, 65% Suprapur, was from Merck; Folin and Ciocalteu's phenol reagent and glucose were from Sigma-Aldrich; absolute ethanol was from Carlo Erba Reagents; hydrogen peroxide was from Fischer Scientific; sodium hypochlorite, sodium hydroxide, iron(III) nitrate, copper nitrate, cadmium nitrate, lead nitrates, tartaric and gallic acids, and fructose were from Panreac. All reagents were p.a. (for analysis) if not stated otherwise. Ultrapure water produced by Merck Millipore Water System (18 MΩ cm^{-1}) was used for solution preparation and sensor washing.

Red table wine from Ribatejo region, Portugal, was purchased from the producer and used for the optimization of sample preparation procedure and development and testing of the measuring protocol for chemical sensors.

2.2. Sample pre-treatment

Wine pre-treatment procedures involving use of various oxidizing agents (hydrogen peroxide, nitric acid and sodium hypochlorite) and heating regimes were tested. List of the conditions used for wine pre-treatment is shown in the Table 1. All trials were made with 15 ml of red wine. Concentrated nitric acid and 30% hydrogen peroxide were used for wine oxidation. After digestion

Table 1
Conditions used for wine digestion.

	Reagents	Wait time	pH adjustment	Digestion conditions
1	HNO_3 (4 ml), H_2O_2 (2 ml)			Microwave
2	HNO_3 (3 ml), H_2O_2 (2 ml)			Wet digestion, 1 h
3	HNO_3 (3 ml), H_2O_2 (2 ml)			Wet digestion, 2 h
4	HNO_3 (3 ml), H_2O_2 (2 ml)			Wet digestion, 3 h
5	HNO_3 (3 ml), H_2O_2 (2 ml)	16 h		Wet digestion, 3 h
6	HNO_3 (3 ml), H_2O_2 (3,5 ml)	44 h		Wet digestion, 3 h
7	HNO_3 (3 ml), H_2O_2 (2 ml)	24 h		Wet digestion, 3 h
8	HNO_3 (3 ml), H_2O_2 (2 ml)	16 h		Wet digestion, 4 h
9	HNO_3 (3 ml), H_2O_2 (2 ml)	24 h		Wet digestion, 4 h
10	H_2O_2 (15 ml)	24 h		Evaporation, ~3 h
11	H_2O_2 (15 ml)	24 h	9	Evaporation, ~3 h
12	NaClO (10 ml)	24 h		
13				Dry ashing at 460°c, 6 h
14	NaClO (10 ml)			Evaporation, 3 h

sample volume was adjusted to 15 ml by ultrapure water and pH was adjusted by addition of NaOH or HNO_3 when necessary.

Dry ashing was carried out in the muffle furnace Selecta (SELECT-HORN) at 460 °C during 6 h. Prior to ashing, wine was dried and carbonized on the heating plate. Ash was first dissolved in a small amount of 0.1 mol L^{-1} nitric acid after which sample volume and pH have been adjusted.

Microwave digestion has been carried out in the Ethos Microsynth System oven (Milestone Microwave Laboratory Systems). The following sequence has been used: 3 min for heating till 85 °C at 800 W; 12 min for heating till 145 °C; 6 min for heating 180 °C;15 min at 180 °C [31]. After the end of the program, digested wine was left to cool in the reactors before it was removed and its volume adjusted to 15 ml.

Degree of wine digestion was evaluated using Fourier Transform mid-infrared Spectroscopy (FT-MIR) and total phenolics' content. FT-MIR-ATR spectra were recorded using Bruker FT-MIR spectrometer ALPHA with ATR sampling accessory in the wave number range 4000–500 cm^{-1} with resolution of 4 cm^{-1} and acquiring 128 scans. Total phenolic content was determined using the Folin–Ciocalteu method [34]. Measurements were made in triplicates.

Content of copper, iron, lead and cadmium in wine was analysed using Inductively Coupled Plasma Mass Spectrometry (ICP-MS) at the Institute of Wine of Douro and Porto, Porto, Portugal.

2.3. *Measurements with sensor array*

Twelve potentiometric chemical sensors with chalcogenide glass and plasticized PVC membranes have been used including four copper-sensitive sensors with chalcogenide glass membranes, of which Cu1 and Cu2 were two conventional copper selective sensors [35] and Cu3 and Cu4 were experimental compositions [36]; three iron(III) sensitive sensors with chalcogenide glass membranes, Fe1 [36], Fe2 and Fe3 [35,37]; one lead and one cadmium sensitive sensors with chalcogenide glass membranes, Pb1 and Cd1, [35], one lead-sensitive, Pb2 [38] and one cross-sensitive to lead and cadmium sensor with plasticized PVC membranes [39]. All polymeric and chalcogenide glass sensors had solid inner contact. All sensors except reference electrode were developed and produced in the Laboratory of Chemical Sensors of St. Petersburg University, Russia. Double junction Ag/AgCl (3 mol L^{-1} KCl) reference electrode and pH glass electrode from Metrohm were used.

Calibration measurements with sensors were made in the individual solutions of iron(III), copper, lead and cadmium, in the mixed model solutions containing copper, lead and cadmium, in model wine solutions containing iron(III) or copper and in wine after digestion, both with and without additions of copper, lead and cadmium.

Calibration measurements with respect to copper were done on the background of 0.01 mol L^{-1} KNO_3 at pH 6 and 3.24, and in the model wine in the concentration range 3×10^{-8}–10^{-4} mol L^{-1}. Composition of the model wine solutions was: ethanol 12 w/w%, tartaric acid 5.8 g L^{-1}, sugars (glucose:fructose 1:1) – 6.4 g L^{-1}, gallic acid – 1277 mg L^{-1} and pH 3.24.

Iron(III) calibration solutions were prepared on the background of 0.01 mol L^{-1} nitric acid and in the model wine in the concentration range 10^{-7}–10^{-3} mol L^{-1} at pH 3.24 and 2. A series of buffer solutions containing citrate was prepared to measure low activity levels of iron(III). Compositions of Fe(III)/citrate buffer solutions are shown in Supplementary Table 1

Calibration measurements in the individual lead and cadmium solutions were carried out on the background of 0.01 mol L^{-1} KNO_3 at pH 6. A series of buffer solutions containing citrate was prepared to measure low activity levels of lead and cadmium. Compositions of citrate buffer solutions are shown in Supplementary Table 1

At least three replicated calibration measurements were run for all metals and conditions. Calibration measurements in the standard solutions prepared on the background of 01 molL^{-1} KNO_3 at pH 6 or 0.01 mol L^{-1} HNO_3 at pH 2 (in the case of Fe) were repeated at least once a week.

Measurements with sensor array comprising 9 sensors were made in mixed model solutions containing Cu, Pb and Cd. Solutions were prepared on the background of 0.01 mol L^{-1} KNO_3 at pH 6. Solution compositions were defined using fractional design using concentration ranges of these three metals typically encountered in wine (Supplementary Table 2). The set of model solutions was measured 4 times during 3 months period.

Measurements with sensor array were made in digested wine with and without additions of iron (III) copper, lead and cadmium. Content of iron(III), copper, lead and cadmium in wine determined by ICP-MS and concentrations of spikes are shown in the Table 2. Wine samples were spiked prior to the digestion. After digestion pH of the wine was adjusted to 2 for iron(III) detection and to 6 for copper, lead and cadmium detection by addition of NaOH. Three replicated measurements were made.

All potentiometric measurements were done using custom-made digital multichannel voltmeter with high input impedance connected to PC for data acquisition. Between measurements sensors were washed with ultrapure water till they reached stable potential. Between measuring sessions sensors were kept in the air.

2.4. *Data processing*

Parameters of the Nernst equation, standard potential and slope, were calculated using ordinary least square regression. Detection limits were determined using procedure recommended by IUPAC [40].

Activities of metal cations in buffer solutions containing citrate and model wine solutions were done using speciation software MINTEQA2 by Center for Exposure Assessment Modeling (CEAM), U.S. Environmental Protection Agency, Office of Research and Development, Environmental Research Laboratory, Athens, Georgia, USA [41].

Calibration models for predicting copper, lead and cadmium concentrations in multicomponent solutions were calculated using Partial Least Square regression (PLS) and back-propagation neural network (BPNN). Calibration models for predicting copper, lead and cadmium concentrations in digested wine were also calculated using BPNN. Models were calculated for each metal

Table 2
Concentrations of iron, copper, lead and cadmium in red wine and in spiked wine samples. Initial concentrations of metals in spiked samples are shown with italic.

	Fe(III) (mmol L^{-1})	Cu (μmol L^{-1})	Pb (μmol L^{-1})	Cd (μmol L^{-1})
	Wine			
	0.0786	*1.57*	*0.097*	*0.009*
	Spiked wine			
1	*0.0786*	*1.57*	0.32	0.08
2	*0.0786*	15.8	100	0.08
3	*0.0786*	40	0.32	0.50
4	*0.0786*	*1.57*	10	1
5	*0.0786*	*1.57*	100	5.0
6	*0.0786*	*1.57*	1000	10
7	*0.0786*	4.0	1	10
8	0.275	*1.57*	*0.097*	*0.009*
9	0.472	*1.57*	*0.097*	*0.009*

individually. PLS models were validated using segmented cross-validation with replicated measurements being always included in either calibration or cross-validation set. Back-propagation network with one hidden layer and hyperbolic tangent sigmoid and linear transfer functions in the hidden and output layers, respectively, was used. Number of neurons in the hidden layer was optimized for each calibration model. Network was trained using Bayesian regularization backpropagation algorithm [42], which affords good generalization without use of monitoring data set. Data were split into calibration and test sets in the ratio 2 to 1 always including replicated measurements in either calibration or cross-validation set. Parameters used for comparison of calibration models were slope and offset of predicted vs. measured curve, root mean square error (RMSE) and adjusted R^2. The latter two parameters were calculated using the following formulas:

$$\mathrm{RMSE}=\sqrt{\frac{\sum_{i=1}^{N}(\hat{y}_i-y_i)^2}{N}},\quad R^2_{adj}=1-\frac{\sum_{i=1}^{N}(\hat{y}_i-y_i)^2}{\sum_{i=1}^{N}(\bar{y}-y_i)^2}\frac{N-1}{N-p-1},$$

where $\hat{y}_i$ is the predicted concentration for the sample i, $\bar{y}$ is the average concentration, y_i is the measured concentration in the sample I, N – total number of samples, and p – number of predictors (estimated calibration model parameters).

PLS were performed using Unscrambler v. 9.7 by CAMO, Norway. Back-propagation ANN was implemented in MATLAB, v. 7.12 (release 2011a) using Neural Network toolbox.

3. Results and discussion

3.1. Sensor responses in the individual metal solutions

Sensor responses to four metals were evaluated in their individual solutions prepared at different backgrounds and pH levels with the aim to evaluate their influence on sensor performance. Metal concentrations were selected to comprise typical ranges encountered in wine according to the literature, which were 0.02–3 mg L^{-1} for copper, 0.24–19.4 mg L^{-1} for iron, 0.001–1.125 mg L^{-1} for lead and 0.1–8.10 μg L^{-1} for cadmium.

Nernstian response and good reproducibility of the sensors in the copper solutions on the background of KNO_3 was confirmed (Fig. 1 and Table 3). Exception was sensor Cu2, which displayed super-Nernstian response and slightly worse reproducibility. However, as it had lowest detection limit of all sensors, it was kept in the array. Detection limits were found to be 2–10 times lower than lowest copper concentration reported in wine and 100 to 500 times lower than maximum allowed concentration. Protons were found to interfere with the sensors' response to Cu at pH levels typical for wine, where slight decrease of the sensitivity, increase of the detection limit and change of standard potential were observed. Significant increase of detection limit was observed in model wine solutions, which was attributed to the formation of complexes between copper and some of the main wine constituents, i.e. sugars and organic acids (Fig. 1a). Taking into account complex formation and plotting sensor response with respect to copper ion activity, sensor responses in KNO_3 at pH 6 and in model wine become co-linear (Fig. 1b). However, detection limit in model wine is still somewhat higher due to the matrix effects.

Response of the iron-selective sensor to Fe(III) at different conditions is shown in Fig. 2. Response characteristics of 3 sensors used for iron detection are shown in Table 4. Super-Nernstian response to Fe(III) was observed in 0.01 mol L^{-1} HNO_3 though detection limits were quite high for all sensors. Increased detection limits of potentiometric sensors are attributed to the contamination of the sensor diffusion layer by the metal cations, which may arise from the carry-over and metal release from the sensor membrane [43,44]. One of the approaches permitting to reduce contamination and consequently, ameliorate detection limits is the use of the solutions buffered with respect to the activity of the metal cation of interest. Buffering is achieved by

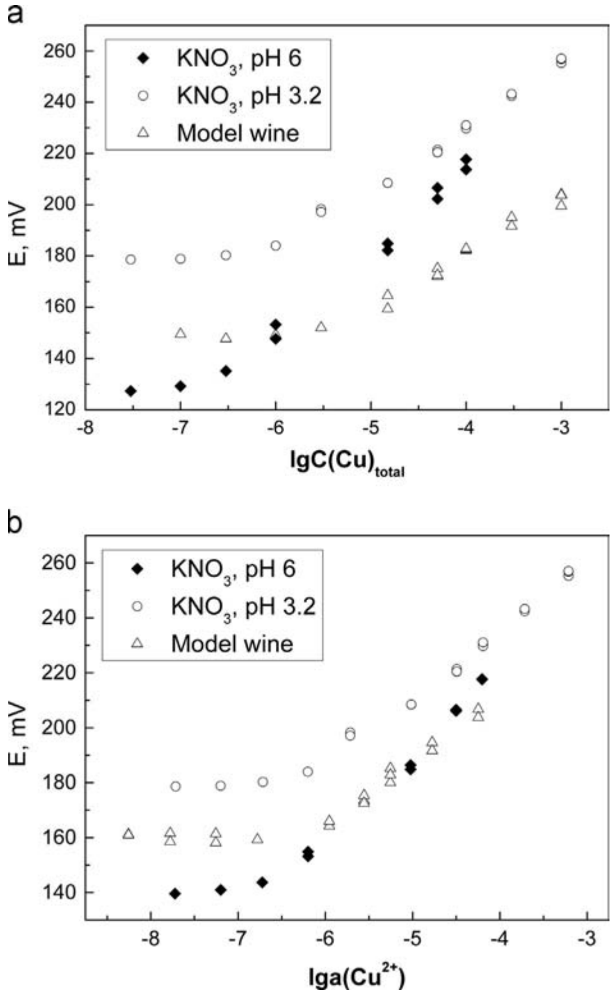

Fig. 1. Response of Cu-selective sensor Cu1 to copper at different conditions: on the background of 0.01 mol L^{-1} KNO_3 with pH 6 and 3.24 and model wine with pH 3.24. Sensor response is plotted vs. total copper concentration in solutions (a) and copper ion activity calculated using MINTEQA2 software (b).

Table 3
Characteristics of sensors used for copper detection at different backgrounds: 0.01 mol L^{-1} KNO_3 with pH 6 and 3.24 and model wine with pH 3.24. E^0 – standard potential, S – slope of the electrode function, DL – low detection limit. Means and standard deviations (in the parentheses) of 4 replicated calibration measurements are shown.

Sensor parameters	Cu1	Cu2	Cu3	Cu4
KNO_3, pH 6				
E^0 (mV)	342 (2)	452 (8)	362 (5)	355 (9)
S (mV/pM)	32 (1)	57 (1)	32 (1)	34 (2)
DL (mg L^{-1})	0.01	0.002	0.009	0.004
KNO_3, pH 3.24				
E^0 (mV)	339 (3)	364 (4)	377 (9)	356 (10)
S (mV/pM)	27 (1)	56 (1)	30 (1)	29 (1)
DL (mg L^{-1})	0.1	0.45	0.11	0.06
Model wine, pH 3.24				
E^0 (mV)	278 (1)	265 (8)	305 (5)	291 (6)
S (mV/pM)	24 (1)	37 (1)	24 (1)	25 (1)
DL (mg L^{-1})	0.36	0.16	0.19	0.11

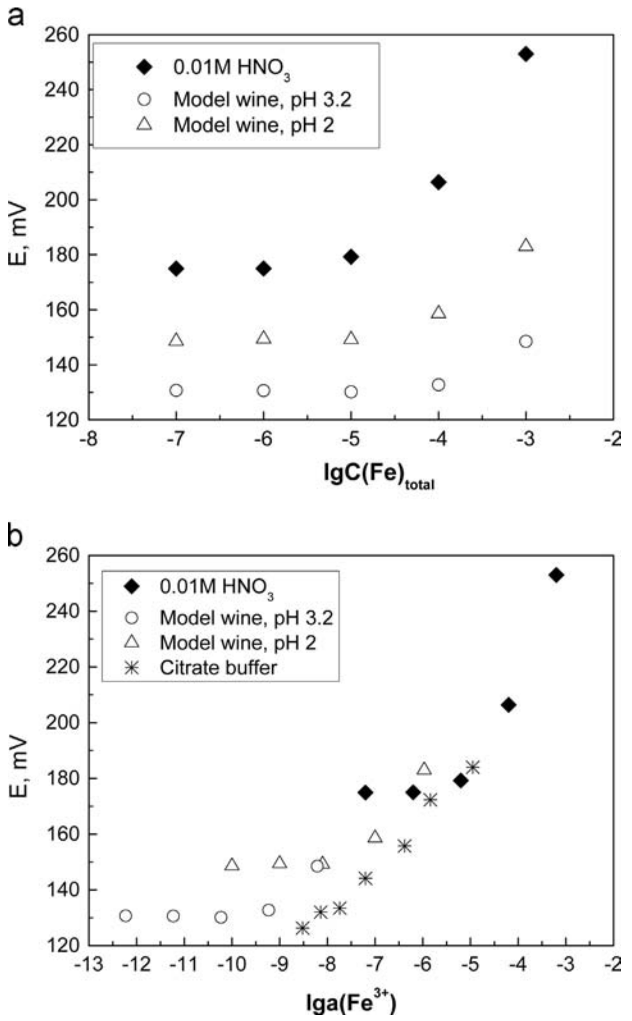

Fig. 2. Response of Fe-selective sensor Fe1 to Fe(III) at different conditions: on the background of 0.01 mol L^{-1} HNO$_3$, citrate buffer and the model wine with pH 3.24 and 2. Sensor response is plotted vs. total iron concentration in solutions (a) and iron(III) ion activity calculated using MINTEQA2 software (b).

Table 4
Characteristics of sensors used for Fe(III) detection at different backgrounds: 0.01 mol L^{-1} HNO$_3$ with pH 2, citrate buffer (see Supplementary Table 1 for composition), model wine with pH 3.24 and 2. E^0 – standard potential, S – slope of the electrode function, DL – low detection limit. Means and standard deviations (in the parentheses) of 4 replicated calibration measurements are shown.

Sensor parameters	Fe1	Fe2	Fe3
HNO$_3$, pH 2			
E^0 (mV)	350 (12)	350 (10)	400 (12)
S (mV/pM)	35 (3)	32 (3)	44 (3)
DL (mg L^{-1})	0.35	0.37	0.37
Citrate buffer			
E^0 (mV)	280 (12)	296 (8)	289 (9)
S (mV/pM)	19 (1)	22 (1)	22 (2)
DL (mg L^{-1})	<0.17	<0.17	<0.17
Model wine, pH 3.24			
E^0 (mV)	197 (12)	182 (5)	179 (8)
S (mV/pM)	16 (3)	10 (2)	13 (2)
DL (mg L^{-1})	1.25	0.44	0.56
Model wine, pH 2			
E^0 (mV)	228 (4)	238 (10)	238 (17)
S (mV/pM)	17 (1)	16 (4)	20 (4)
DL (mg L^{-1})	0.22	0.56	0.56

addition of complexing agent. Very low response to Fe(III) and only at high concentrations was observed in model wine solutions due to the complex formation (Fig. 2a).

Thus, measurements were made in citrate buffers that had high total concentration of Fe(III) but low activity due to the presence of the complexing agent – citrate (Supplementary Table 1). Detection limit of iron-sensitive sensors in citrate buffer solutions was found to be very low: below 0.17 μg L^{-1}, which is more than 1000 times lower than possible concentrations in wine. Response to Fe(III) in citrate buffer is aligned with response in HNO$_3$ but slope of the electrode function is lower (Fig. 2). Non-Nernstian response of chalcogenide glass sensors to Fe(III) with lower slopes at lower concentrations has been reported earlier [35] and in fact such behaviour is known for the sensors, whose response mechanism is based on both ion-exchange and redox processes [45]. Increase of pH from 2 to 3.24 and presence of the main wine components had negative effect on sensor performance resulting in the decrease of the sensitivity and increase of detection limits (Table 4).

Nernstian response to lead ions and sub-Nernstian response to cadmium were observed using KNO$_3$ as a background electrolyte (Table 5). These values are in agreement with literature data [35]. Though, detection limits for both lead and cadmium were lower than maximum allowed concentrations of these metals in wine, they were not low enough to detect these metals in the entire concentration range typically encountered in wine. Similarly to the iron-sensitive electrodes, increase of the detection limits could be caused by the contamination of solutions from reagents or sensor membranes. Thus, measurements were carried in the buffer solutions with relatively high concentrations of cadmium or lead and complexing agent citrate leading to very low activities of metal ions (Supplementary Table 1). Responses of the sensors to Pb^{2+} and Cd^{2+} in the citrate buffers and in KNO$_3$ solutions were collinear indicating the same response mechanism. Detection limits of lead and cadmium sensitive sensors in citrate buffer solutions were found to be very low: below 0.39 and 0.04 μg L^{-1}, respectively. These results demonstrate that the set of sensors could be applied to the quantification of lead and cadmium in wine.

In conclusion, studied sensors were found suitable for the detection of Cu, Fe(III), Pb and Cd at the concentration levels typically present in wine. However, pH adjustment and digestion of the organic matter present in wine samples are necessary to prior to the detection of total metal content.

3.2. *Optimization of the sample preparation procedure*

List of used wine digestion/pre-treatment procedures is shown in the Table 1. These conditions represent variations of the

Table 5
Characteristics of sensors in the individual solutions of Pb and Cd at different backgrounds: 0.01 mol L^{-1} KNO$_3$ with pH 6 and citrate buffer (see Table 4 for compositions). E^0 – standard potential, S – slope of the electrode function, DL – low detection limit. Means and standard deviations (in the parentheses) of 4 replicated calibration measurements are shown.

Sensor parameters	C1 (in Pb solutions)	Pb1	Pb2	C1 (in Cd solutions)	Cd
KNO$_3$, pH 6					
E^0 (mV)	387 (2)	-34 (4)	422 (2)	353 (11)	-140 (7)
S (mV/pM)	34 (1)	28 (1)	32(1)	29 (2)	24 (2)
DL (mg L^{-1})	45	19	36	13	7
Buffer					
E^0 (mV)	240 (22)	-59 (3)	316 (3)	-2 (9)	-152 (8)
S (mV/pM)	20 (3)	22 (1)	39 (2)	42 (2)	33 (1)
DL (mg L^{-1})	<0.39	<0.39	<0.39	0.45	<0.04

microwave, digestion, wet digestion and dry ashing methods, which were described in the literature for wine [13,29–33]. Various digestion methods were evaluated with two-fold aim: achieve complete decomposition of organic compounds while using as simple procedure as possible, which would not require expensive equipment and could be carried out at the winemaking site. It is important to note that due to high organic matter content in wine, wine digestion prior to analysis using spectroscopic techniques including ICP-MS is often recommended, as simple dilution was demonstrated to lead to erroneous results [29].

Effectiveness of the digestion of organic matter in wine was assessed using FT-MIR-ATR spectroscopy in the fingerprint region (i.e. 1200–900 cm^{-1}) and content of total phenols. FT-MIR-ATR spectra and total phenols content in wine before and after different digestion procedures are shown in Figs. 3 and 4, respectively. The most effective method of wine digestion was found to be dry ashing (13). None of other procedures was equally effective for the simultaneous decomposition of alcohols, sugars, organic acids and phenolic compounds. For example, about 140 mg L^{-1} of phenolics were still present after microwave assisted digestion (1), which is often recommended for the pre-treatment of wine samples. FT-MIR spectra of wine after this treatment did not show presence of organic substances (Fig. 3). Some of the wet digestion procedures, e.g. 9 and 11, allowed significant reduction of the content of both sugars and organic acids and phenolic compounds, though not their complete removal. Thus, dry ashing was further used for wine digestion.

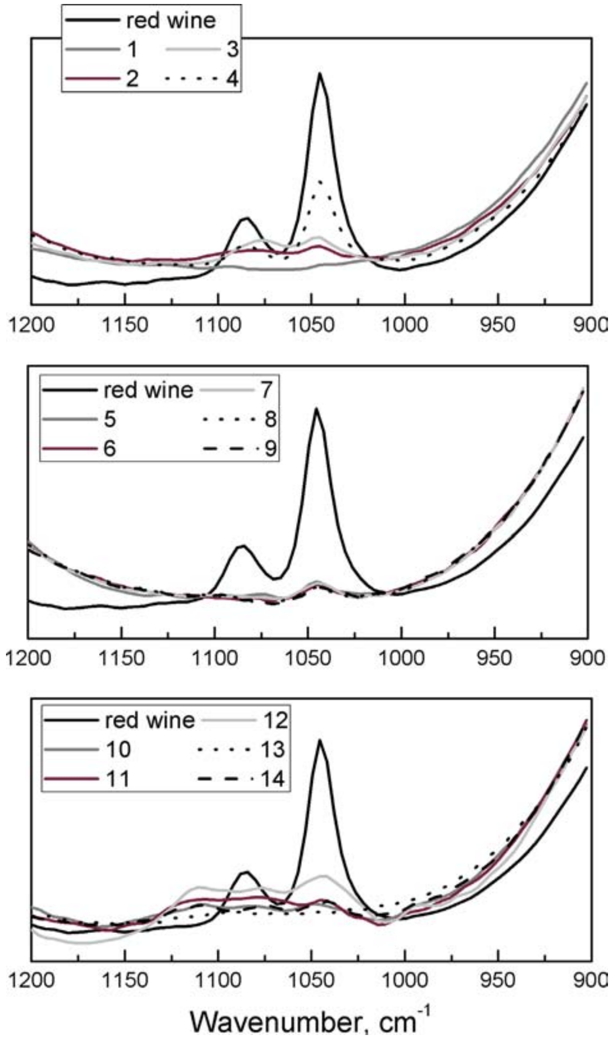

Fig. 3. FT-MIR spectra of red wine before and after different treatments (see Table 1 for the treatment codes).

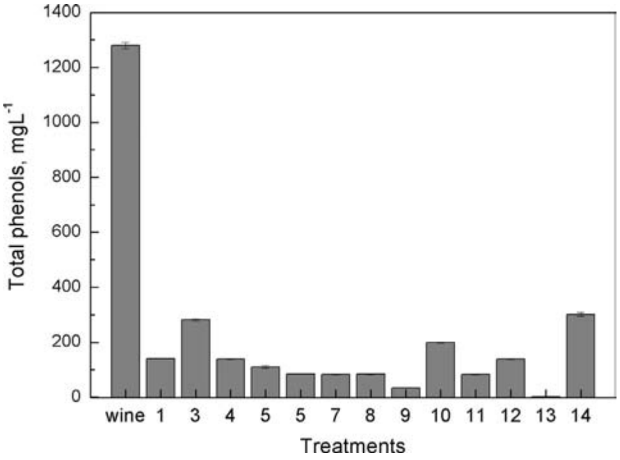

Fig. 4. Content of total phenols (expressed as mg L^{-1} of gallic acid) in red wine before and after different digestion treatments (see Table 1 for treatment codes).

3.3. Determination of copper, lead and copper in mixed model solutions

Iron content in wine is relatively high and iron-sensitive sensors are sufficiently selective to iron (III) in the presence of the other transition metals or other compounds that may be present in digested wine. For example, log K_{sel} in the presence of copper is -3 for the sensor Fe1, while selectivity coefficients to lead and cadmium are usually not reported as these ions do not interfere with response to Fe^{3+} [37]. Therefore, it was expected that sensors can be used to measure iron in digested wine directly (see results in the next section). The case is different with copper, lead and cadmium that are present at lower concentration, at which sensor responses become less reproducible and non-linear. Another factor leading to the non-linearity of the responses of cadmium and lead-sensitive sensors is their cross-sensitivity [35,36,38,39]. Thus, use of sensor array and multivariate calibration was considered necessary for detection of copper, lead and cadmium.

Calibration models were calculated using PLS regression and back-propagation neural net. First, optimization of the neural network architecture namely number of neurons in the hidden layer has been done. Two parameters were used as optimization criteria: Root Mean Square Error (RMSE) and adjusted R^2. Adjusted R^2 is a R^2 corrected for the number of explanatory terms in the model and thus, penalizes models with high number of parameters. While R^2 usually increases when a new term is added to the model, the adjusted R^2 increases only if the added term improves the model more than would be expected by chance. This property is useful when comparing performance of neural networks with different architectures: addition of even one hidden neuron leads to drastic increase of number of estimated model parameters (10 more in the case of 9 input variables) and also increases probability of overfitting. Values of RMSE and adjusted R^2 for neural nets with 1–4 hidden neurons for the determination of copper, lead and cadmium are shown in Fig. 5a, b and c, respectively. Networks with minimum RMSE and maximum adjusted R^2 were the ones with 3 hidden neurons for copper and cadmium prediction and with 2 neurons for lead prediction. Small difference between adjusted R^2 values for calibration and prediction indicates that selected models were not overfitted.

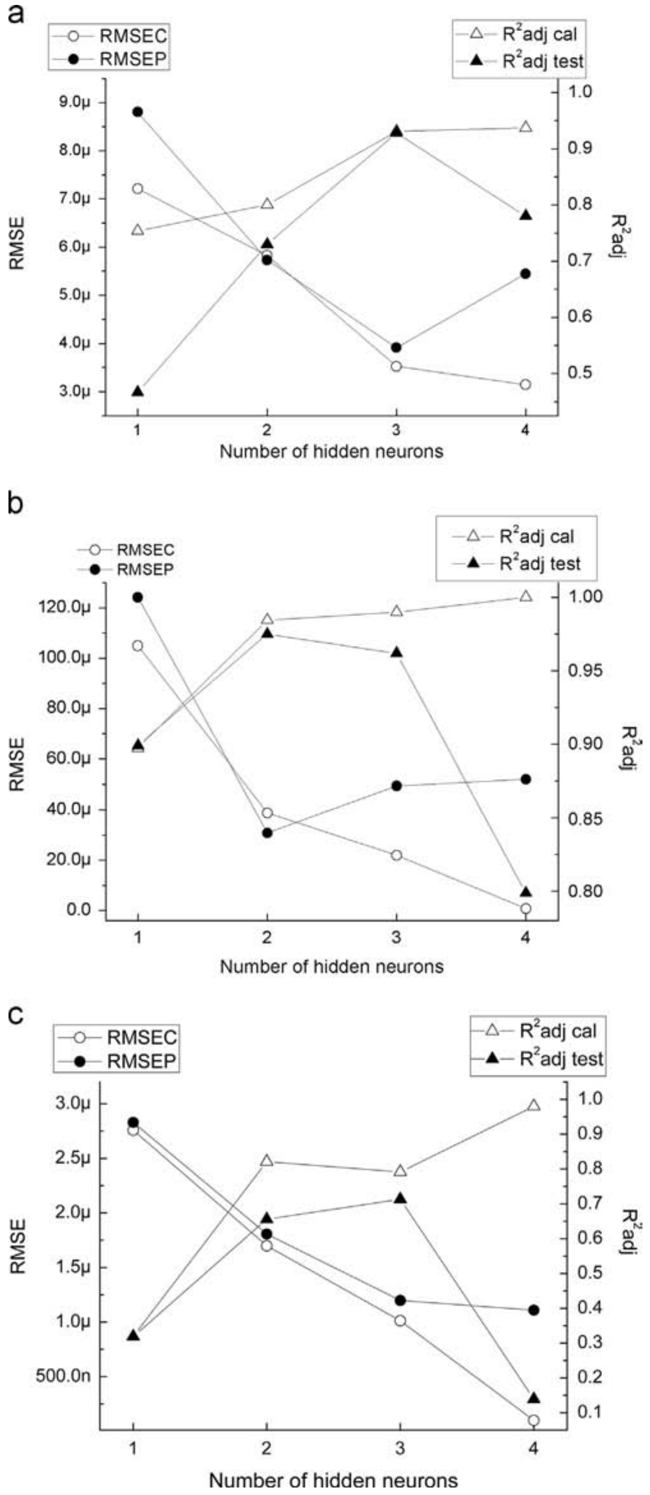

Fig. 5. Parameters of BPNN calibration models (Root Mean Square Error (RMSE) and adjusted R^2) with different number of hidden neurons for the quantification of (a) copper, (b) lead and (c) cadmium.

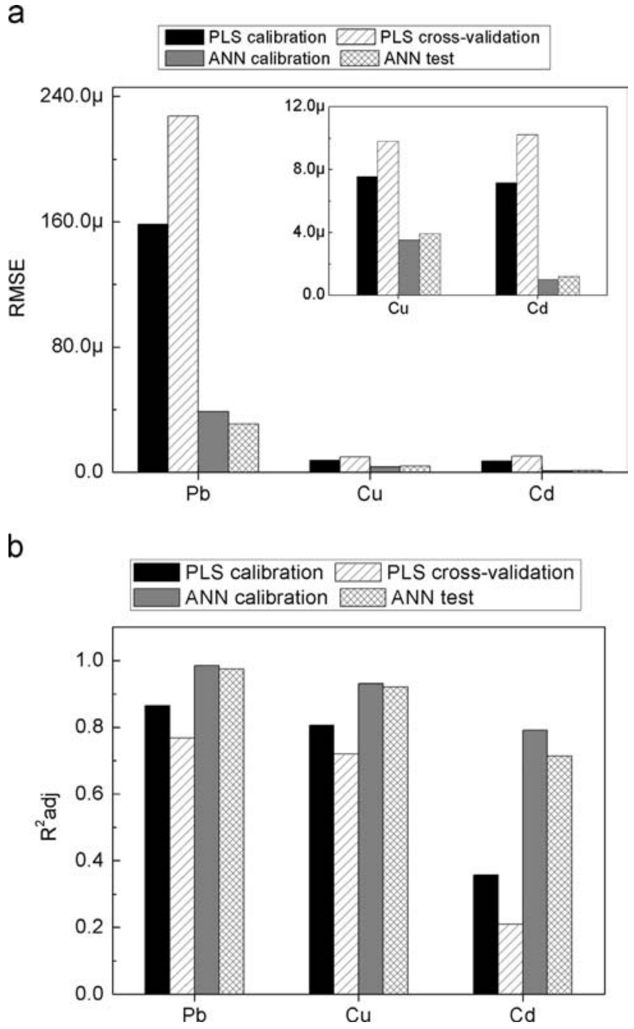

Fig. 6. Comparison of the performance of PLS and BPNN calibration models for the prediction of copper, lead and cadmium (a) Root Mean Square Errors (RMSE) and (b) adjusted R^2 values.

Comparison of the performance of PLS regression and optimized BPNN models is shown in Fig. 6 and Supplementary Table 3 Prediction of concentrations of copper and lead was possible using PLS models, though with high prediction errors in the case of lead. PLS model for cadmium prediction was unusable as it had very low adjusted R^2 value. This indicates presence of non-linearity in responses of cadmium-sensitive sensors, which could not be taken into account by the method like PLS regression. Indeed, nonlinear calibration models have shown lower prediction errors and higher adjusted R^2 values, thus, proving to be more adequate technique for modeling sensor responses in this case. Higher degree of non-linearity of responses to cadmium is a consequence of the lower selectivity of cadmium-sensitive sensors compared to lead and copper-sensitive ones. Selectivity coefficients of the cadmium-sensitive sensors with chalcogenide glass and PVC plasticized membranes are 3.9 and -2 to copper and 1.8 and -1.6 to lead, respectively [35,38]. Lead-sensitive sensors are more selective in the presence of cadmium with log K_{sel} of -1.6 and -3.7 for the sensors with chalcogenide glass and plasticized PVC membranes, respectively [35,39].

Non-linearity of the sensor responses can be assessed by plotting output of hidden neurons vs. their input (Supplementary Fig. 1a–f). Activation of hidden neurons demonstrates that sensor responses to all three ions had both linear and non-linear segments. Interestingly, though copper-sensitive sensors are more selective compared to lead and cadmium-sensitive one, response of the sensor array to lead ions was more linear (Fig. 1d and e).

3.4. Determination of iron(III), copper, lead and cadmium total content in digested wine

Determination of iron(III) concentration in digested wine samples was possible using single iron-sensitive sensor and a

Table 6
Parameters of the calibration models calculated using back-propagation neural network with optimal configurations (see text for more details) for the determination of copper, lead and cadmium in digested wine samples: root mean square error (RMSE), slope and offset of predicted vs. measured curve and adjusted R^2.

Metal	Cu		Pb		Cd	
	Calibration	Validation	Calibration	Validation	Calibration	Validation
Slope	0.96	1.02	0.90	1.35	0.99	0.57
Offset ($\times 10^{-6}$)	0.3	0.8	40	55	-0.2	-0.04
RMSE (μmol L^{-1})	2	2	100	150	1	1
R^2 adj	0.89	0.84	0.88	0.71	0.67	0.65

calibration curve produced in the model solutions. RMSEP was found to be 0.05 mmol L^{-1} in the concentration range from 0.0786 (iron concentration in wine determined by ICP-MS) and 0.472 mmol L^{-1}.

Prediction of copper, lead and cadmium content in digested wine samples using neural network models developed using model solutions was not successful as RMSEP of 12, 860 and 9 μmol L^{-1} for copper, lead and cadmium, respectively. These errors are 3, 21 and 7.5 times higher that RMSEP values obtained with model solutions indicating significant effect of residual matrix compounds in digested wine on the sensor responses. Therefore, a set of digested wine samples spiked with different levels of copper, lead and cadmium has been measured and used for the neural network calibration testing (see Table 2 for the concentration levels). This allowed to significantly improve accuracy of metal concentration prediction, particularly in the case of copper of cadmium with RMSEP values of their determination being close to the ones obtained in model solutions (Table 6).

4. Conclusions

Capability of a sensor array based on the potentiometric chemical sensors with chalcogenide glass and plasticized PVC membranes to detect Fe(III), Cu, Pb and Cd in digested wine has been demonstrated. Iron could be detected using discrete iron-selective sensors and calibration curves produced in the model solutions. Copper, lead and cadmium could be detected simultaneously using sensor array and calibration models calculated using back-propagation neural network. Various procedures of sample preparation have been considered. Dry ashing was identified as an optimal one, allowing most complete digestion of organic matter of wine.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.talanta.2014.04.030.

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