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Optimization of digestion procedure for the determination of nickel in wine by differential-pulse adsorptive stripping voltammetry

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A method for the determination of nickel in wine by differential-pulse adsorptive stripping voltammetry using dimethylglyoxime as complexing agent was optimised using experimental design methodology. In order to destroy organic matter, two different methods of treating the sample were considered. The UV irradiation method was selected and optimised for a smaller degree of uncertainty. The recovery factor obtained with five replicates was 1.02 ± 0.05.

Keywords: *Nickel; wine; experimental design; differential-pulse voltammetry; UV digestion; combined uncertainty*

Official methods for the determination of heavy metals in wine produced by the Office International de la Vigne et du Vin and the American Society of Enologists are essentially based on atomic absorption spectrometry. Electrochemical techniques have rarely been used in wine analysis owing to the lack of reproducibility in some cases and to the difficulty in automation in others. However, the high sensitivity of the latter techniques, combined with inexpensive instrumentation, makes them eminently suited for this task.

There are few reliable data in the literature concerning nickel content in wines. Eschenauer[1] found nickel concentrations ranging between 0 and 1.87×10^{-5} mol dm^{-3}. Meranger and Sommers,[2] after analysing 24 wines from different regions, found the nickel content to vary between 8.52×10^{-7} and 2.89×10^{-6} mol dm^{-3}. Nickel is present in wines owing to the use of Ni-containing stainless-steel containers for wine fermentation and storage in modern cellar technology.

In this paper, the determination of nickel in wine by differential-pulse adsorptive stripping voltammetry (DPAdSV) based on adsorption of the Ni–dimethylglyoxime complex is reported. With the aim of guaranteeing the analytical results, an optimization process using a 2^3 design was carried out in which factors relevant to the DPAdSV technique intervene. Special attention was given to the choice of a suitable digestion procedure, two differents methods of mineralization of the wine sample being examined.

The analytical results were expressed to take into account the ISO Guide[3] for the expression of uncertainty in measurement, which establishes general rules for the evaluation and expressing uncertainty in measurement, and the Eurachem Guide,[4] which shows how the concepts in the ISO Guide may be applied in chemical measurements to take into account all possible sources of uncertainty implicated in the proposed procedure.

Experimental

Reagents

Stock standard solutions of nickel were prepared by dissolving the appropriate amount of nickel powder (analytical-reagent grade, Merck, Darmstadt, Germany) in the minimum volume of HNO_3 (Suprapur grade, Merck) and diluting to volume with de-ionized water. Ammonia–ammonium chloride (Suprapur grade, Merck) buffer was used as the supporting electrolyte. Hydrogen peroxide used in the digestion was of analytical-reagent grade (30%; Merck) and H_2SO_4 was of Suprapur grade (96%; Merck). All solutions were prepared with de-ionized water obtained with a Barnstead (Dubuque, IA) NANO Pure II System. A 10^{-2} mol dm^{-3} dimethylglyoxime (DMG) solution was prepared by dissolving the appropriate amount of analytical-reagent grade compound (Merck) in 96% ethanol (analytical-reagent grade, Merck). Samples of commercial wines were used.

Apparatus

Sample digestion in acidic media was performed in a Selecta Bloc Digest 6, (Barcelona, Spain) and the UV photolysis of the samples in a Model 705 UV digester (Metrohm, Herisau, Switzerland).

Voltammetric measurements were carried out using a Metrohm Model 646 VA processor and a Model 647 VA electrode stand with a multimode electrode (MME) operating in the hanging mercury drop electrode (HMDE) mode. An Ag/AgCl, 3 M KCl reference electrode and a platinum wire auxiliary electrode were used. A Metrohm automatic 665 Model Dosimat burette was attached to the system.

The pH of the solution was measured with a Crison Model 2002, (Barcelona, Spain) pH meter.

Data analysis was carried out with the packages Statgraphics[5] and Progress.[6] All calculations were performed on a Tandon 486/33 PC.

Procedure

Voltammetric measurements were made using the following procedure: to 10 ml of wine sample, 10 ml buffer and the required amount of DMG to achieve the DMG concentration (C_{DMG}) indicated in each case by the experimental design were added. The pH was set to 8.5 in all experiments. Once the solution had been deoxygenated, the stirrer was connected and deposition began according to a time and potential determined for each experiment. When the time had elapsed, the stirrer was switched off and the solution left to settle for an equilibrium time of 10 s. The voltammogram was then recorded by making a cathodic sweep from the deposition potential (initial potential) to −1.1 V (final potential). Four successive sweeps were made from the same solution and the mean value was used in the calculations.

The following values for the experimental parameters were obtained in previous work[7] to give the most reproducible signal: mercury drop size, 0.40 mm^2; stirring rate in the accumulation period, 1290 rev min^{-1}; and amplitude, staircase size and duration of the pulse in the staircase potential sweep, −62 mV, 4 mV and 500 ms, respectively.

Results and discussion

Procedure without digestion

The voltammetric determination of trace elements normally involves very small current responses and this explains the importance of optimising all those parameters which might have an influence on the measured current. Prior voltammetric sweeps on a sample of wine in basic media give signals that are difficult to quantify. However, the addition of a small amount of nickel to the sample permits the observation of a well defined voltammetric peak at a potential of −0.9 V. One can assume from this that the initial experimental conditions were not adequate for the detection of small amounts of nickel in wine, hence the need to optimize the procedure.

As is known in the DPAdSV technique, the response obtained, i_p, is notably influenced by variables such as the time, t_{dep}, and potential, E_{dep}, of deposition and the concentration of the complexing agent (DMG), C_{DMG}. The experimental design was used as a tool for optimisation. A 2^3 factorial design[8–11] was chosen for this stage, its purpose being to arrange the factors and their interactions according to their influence on the peak current. These experiments were carried out on a sample of commercial wine to which a small amount of nickel was added (9×10^{-8} mol dm^{-3} for 10 ml of wine sample + 10 ml of buffer) in order to obtain a measurable signal.

The values which correspond to the high (+) and low (−) levels and to the central point (0) for each factor are the following:

$C_{DMG}(+) = 7.00 \times 10^{-4}$ mol dm^{-3} $\quad E_{dep}(+) = -850$ mV, $t_{dep}(+) = 180$ s

$C_{DMG}(-) = 2.50 \times 10^{-4}$ mol dm^{-3} $\quad E_{dep}(-) = -400$ mV, $t_{dep}(-) = 15$ s

$C_{DMG}(0) = 4.75 \times 10^{-4}$ mol dm^{-3} $\quad E_{dep}(0) = -625$ mV, $t_{dep}(0) = 98$ s

Table 1 shows the mean peak current values obtained with four sweeps being made of each. The analysis of the results from the ANOVA is presented in Table 2. It can be seen that the influential factors in the measurements of the peak current are deposition time, deposition potential and the concentration of DMG. The interactions between factors are not significant. Observation of the response surfaces in Fig. 1 indicates that the peak current is improved by using short deposition times, although times less than 15 s do not seem advisable. Equally, the signal improves with deposition potentials far from the peak potential, although the choice of more positive deposition potentials may cause problems when working with complex samples. For the concentration of DMG, an intermediate value was chosen to ensure always there was an excess of the complexing agent with respect to the nickel concentration in order to avoid problems in later performances of the standard addition method.

In accordance with the above discussion, the following experimental conditions were chosen: $C_{DMG} = 4.75 \times 10^{-4}$ mol dm^{-3}, $E_{dep} = -400$ mV and $t_{dep} = 15$ s.

Using this design, the peak current, i_p, was improved approximately threefold, giving easily quantifiable signals, as can be seen in Fig. 2, which shows the voltammograms of a sample of three different commercial wines which had not undergone any previous treatment. It also shows three successive additions of nickel to these samples. In all cases a well defined peak can be seen.

Digestion procedures

Once the optimum parameters for the analysis had been chosen, a calibration by standard additions was carried out. As is known, wine contains dissolved organic substances which interfere strongly in the determination of heavy metals. If the level of organic matter is sufficiently high, then prior treatment of the

Table 2 ANOVA with the data in Table 1

Effect	*SS**	*DF**	*MS**	F_{ratio}*	P_{level}*
A: C_{DMG}	98.59	1	98.59	31.40	0.030†
B: t_{dep}	475.55	1	475.55	151.46	0.006†
C: E_{dep}	64.35	1	64.35	20.50	0.045†
AB	0.21	1	0.29	0.07	0.823
AC	0.06	1	0.06	0.02	0.903
BC	20.49	1	20.49	6.53	0.125
Residuals	6.28	2	3.14		
Total	665.54	8			

* *SS*, sum of squares; *DF*, degrees of freedom; *MS*, mean squares; P_{level}, probability level. † Significant factor at $\alpha = 0.05$. F_{ratio}: MS_{factor}/MS_{error}.

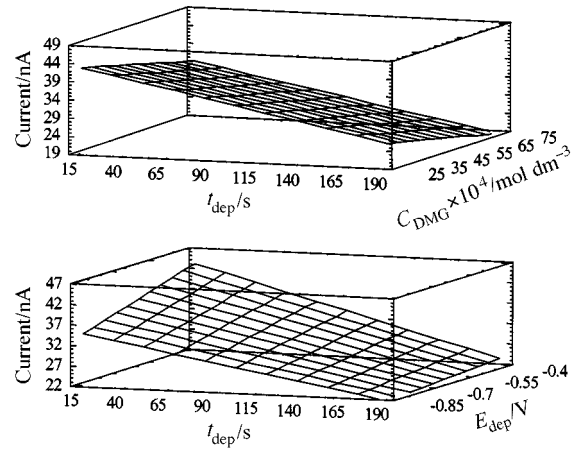

Fig. 1 Response surface for the 2^3 design (Table 1) for deposition time and DMG concentration and time and potential deposition.

Table 1 Results of the 2^3 experimental designs for optimization of variables in determination of nickel in wine by DPAdSV

C_{DMG}/M	t_{dep}/s	E_{dep}/mV	i_1/nA	i_2/nA	i_3/nA	i_4/nA	i_m/nA*
7×10^{-4}	180	−850	18.64	17.97	18.34	17.78	18.18
7×10^{-4}	180	−400	19.79	21.21	22.32	24.25	21.89
7×10^{-4}	15	−850	30.25	31.97	32.01	31.73	31.49
7×10^{-4}	15	−400	37.64	39.26	38.78	39.43	38.77
2.5×10^{-4}	180	−850	27.34	26.31	25.71	25.11	26.12
2.5×10^{-4}	180	−400	27.87	27.50	27.36	27.05	27.35
2.5×10^{-4}	15	−850	37.49	37.72	36.70	37.08	37.25
2.5×10^{-4}	15	−400	44.65	48.17	48.81	49.20	47.71
4.75×10^{-4}	98	−625	29.86	31.20	34.23	35.52	32.70

* i_m is the mean value of the four sweeps i_1, i_2, i_3 and i_4.

sample has to be applied in order to decompose the organic dissolved matter binding Ni^{II}. This results in the liberation of the nickel ion and an increase in the intensity of the voltammetric peak, thus obtaining the total metal *versus* the labile metal when the wine is analysed without previous digestion.[12]

In order to destroy the organic matter, two different procedures for sample digestion were considered. In procedure A, samples of 10 ml of wine were transferred to digestion flasks and 0.5 ml of H_2SO_4 was added to each. The samples were heated to dryness for about 1 h at 280 °C, then 1.5 ml of H_2O_2 was added to each of the samples and the latter were heated for a further 1 h at the same temperature, thus removing the black residue formed in the previous stage. Finally, 1.5 ml of water were added to the samples, which were heated for 1 h at a maximum temperature of 280 °C. Procedure B involved UV irradiation of 3 ml samples of wine with 1.5 ml of H_2O_2, for about 30 min.

The repeatability of the procedure was studied by carrying out six replicate assays with procedure A and another six replicate assays with procedure B. The digested samples of wine were then analysed by DPAdSV. The RSD was 0.21 (n = 6) for procedure A and 0.08 (n = 6) for procedure B, indicating that the repeatability of the latter procedure was better. Furthermore, procedure B is also much faster. It was therefore selected for subsequent experiments.

Once procedure B had been chosen for the digestion of the sample, optimization of the parameters that influence this procedure was carried out using a central composite design, in order to analyse the response surface obtained. The factors to be studied were the amount of H_2O_2 added to the sample and the digestion time. Table 3 shows the average of the voltammetric peaks measured, corrected by the corresponding blanks in the different conditions of the design. The ANOVA (Table 4) shows that there are only digestion time (P_{actual} = 0.033) and amount of H_2O_2 added (P_{actual} = 0.004) effects, these factors squared and their interactions not being influential. The maximum detected by observing the response surface led us to select the following experimental conditions: amount of H_2O_2 = 1.5 ml and digestion time = 15 min. Volumes of H_2O_2

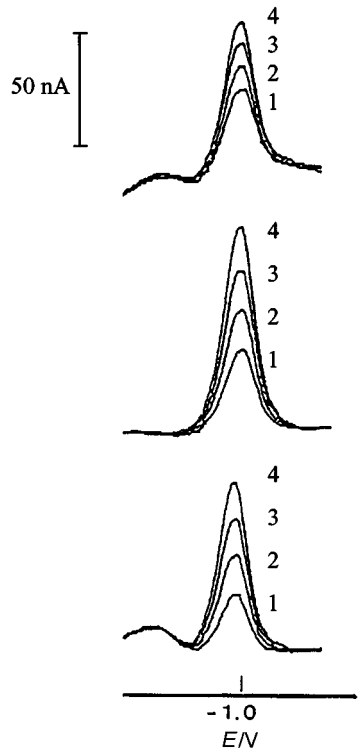

Fig. 2 Differential-pulse voltammograms of nickel in wine without digestion. C_{DMG} = 4.75 × 10^{-4} mol dm^{-3}; E_{dep} = −0.4 V; t_{dep} = 15 s. Ni^{II} concentration added: 1, 0; 2, 4.69 × 10^{-7}; 3, 9.35 × 10^{-7}; 4, 1.39 × 10^{-6} mol dm^{-3}.

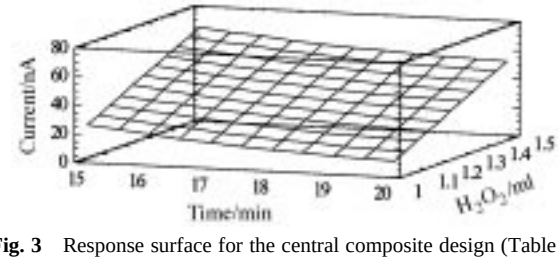

Fig. 3 Response surface for the central composite design (Table 3).

Table 3 Results of the central composite design for optimization of variables in UV irridation digestion

Time/ min	H_2O_2/ ml	i_1/nA	i_2/nA	i_3/nA	i_4/nA	i_m/nA*
17.50	1.25	37.50	37.20	39.07	36.95	37.68
17.50	1.60	62.53	59.97	61.21	64.04	61.94
17.50	0.89	11.35	11.51	12.22	12.64	11.93
15.00	1.50	68.83	69.26	67.78	65.25	67.78
15.00	1.00	20.17	20.70	20.94	21.45	20.82
17.50	1.25	33.27	34.20	27.93	34.61	32.50
20.00	1.00	8.23	7.96	8.32	8.24	8.19
13.90	1.25	54.37	54.94	54.44	52.59	54.08
21.03	1.25	28.92	32.05	29.81	29.91	30.17
20.00	1.50	56.54	61.28	55.70	53.22	56.68
17.50	1.25	44.46	39.12	37.37	38.51	39.86

* i_m is the mean value of the four sweeps i_1, i_2, i_3 and i_4.

Table 4 ANOVA with the data in Table 3

Effect	*SS**	*DF**	*MS**	F_{ratio}*	P_{level}*
A: time	413.9111	1	413.9111	28.96	0.033†
B: H_2O_2	3451.7594	1	3451.7594	241.51	0.004†
AB	0.5852	1	0.5852	0.04	0.860
AA	33.3966	1	33.3966	2.34	0.266
BB	0.1503	1	0.1503	0.01	0.928
Lack of fit	9.8305	3	30.6102	2.14	0.334
Pure error	28.5848	2	14.2924		
Total	4024.8370	10			

* See Table 2. † Significant factor at α = 0.05. R^2 = 0.970.

Table 5 Results of recovery assays of spiked samples without and with digestion. $C_{Ni^{II}}$ added, 10^{-7} mol dm^{-3}

	C_{Ni} found/mol dm^{-3}		Recovery with digestion (%)
	Without digestion	With digestion	
	1.048 × 10^{-7}	1.1570 × 10^{-7}	115.70
	1.011 × 10^{-7}	0.9826 × 10^{-7}	98.26
	1.009 × 10^{-7}	0.9392 × 10^{-7}	93.92
	1.061 × 10^{-7}	1.1457 × 10^{-7}	114.57
	1.098 × 10^{-7}	0.9549 × 10^{-7}	95.49
$\bar{x}$	1.048 × 10^{-7}	0.9826 × 10^{-7}	98.26
s	3.75 × 10^{-9}	1.0660 × 10^{-8}	10.66
RSD	0.03	0.10	0.10

Table 6 Set of parameters in the standard calibration of nickel in wine without and with digestion

No.	Intercept	Slope/ 10^{-8} dm^3 mol^{-1}	ρ	s_{yx}	$(C_{Ni})_{cal.}$/ 10^{-7} mol dm^{-3}	$(C_{Ni})^*$/ 10^{-7} mol dm^{-3}
Without digestion—						
1	4.651	0.8021	0.998	0.5592	0.579	1.245
2	4.892	0.9225	0.998	0.5838	0.530	1.139
3	5.793	0.8980	0.998	0.6905	0.645	1.387
With digestion—						
1	63.622	5.2173	0.998	5.9950	1.219	2.621
2	67.411	5.9790	0.999	3.1458	1.127	2.423
3	73.669	6.5629	0.998	7.5766	1.122	2.412
4	60.452	6.1792	0.999	5.2892	0.978	2.103
5	59.959	5.6879	0.999	2.6478	1.054	2.266
6	66.248	6.1611	0.999	1.7652	1.075	2.311

* Ni concentration taking into account dilution of sample.

above 1.5 ml and digestion times below 14 s caused serious interference when quantifying the signal.

Recovery

In order to express the quantitative results for nickel concentration correctly, a recovery study was performed. For this, 10 spiked samples of 10^{-7} mol dm^{-3} Ni^{II} were analysed by DPAdSV. Five of them were not previously digested whereas the other five were submitted to UV irradiation under the optimum conditions of the design indicated above. The amounts of nickel obtained by standard additions in each of the two situations are shown in Table 5. The mean recovery for five samples is 98.26% with an RSD of 10.66/98.26 (of 98.26%). This leads to an estimated value of the recovery factor (F) of

$$F = \left(\frac{100}{98.26}\right) \pm \frac{(10.66/98.26)}{\sqrt{5}}$$
$$= 1.02 \pm 0.05.$$

Determination of nickel in wine

In order to highlight the complexing effect that the organic matter has on the nickel, a study was performed on samples of wine that had undergone no previous treatment and also samples that had been subjected to UV irradiation.

When obtaining the calibration lines, the criterion of the least median squares regression (LMS)[6] was taken into account to detect anomalous points, whether they be 'outlier' or 'leverage'. The anomalous points detected were eliminated and a regression based on the least-squares (LS) criterion was carried out, to obtain the optimum precision and accuracy of both the slope and intercept. This strategy has been used successfully in other calibration problems.[13–14]

To carry out the determination of nickel in wine, additions of 100 μl of 10^{-5} mol dm^{-3} Ni^{II} were made to samples of commercial white wine (without and with digestion). Calibration parameters and the nickel content in wine, for the replicates of the different calibrations for the two procedures, are summarised in Tables 6 and 7. According to the Eurochem Guide,[4] the nickel concentration in wine is given by the following expressions:

(*i*) without digestion: $C_{Ni\ wine} = C_{Ni\ found} \pm u$ where the uncertainty (u) is

$$u = \frac{s}{\sqrt{n}} \times k$$

$C_{Ni\ wine} = 1.26 \times 10^{-7} \pm 1.43 \times 10^{-8}$ ($k = 2$) mol dm^{-3}

(*ii*) with digestion: $C_{Ni\ wine} = C_{Ni\ found} \times F \pm u_T$, where the combined uncertainty (u_T) is

Table 7 Statistical summary of the determination of nickel in wine for different procedures

Parameter	Without digestion	With digestion: First day	With digestion: Second day	With digestion: First and second day
Sample size	3	3	3	6
Average/ mol dm^{-3}	1.26×10^{-7}	2.48×10^{-7}	2.23×10^{-7}	2.35×10^{-7}
s/mol dm^{-3}	1.24×10^{-8}	1.17×10^{-8}	1.09×10^{-8}	1.74×10^{-8}
RSD	9.87×10^{-2}	4.70×10^{-2}	4.90×10^{-2}	7.40×10^{-2}

$$u_T = s_{total} \times k$$

where

$$s_{total} = (RSD)_{total} \times \bar{x}$$
$$(RSD)_{total} = \sqrt{(RSD)^2_{Ni} + (RSD)^2_{Factor}}$$

where $(RSD)_{Ni}$ is the RSD of nickel calibration (7.40×10^{-2}) and $(RSD)_{factor}$ is the RSD of the recovery factor (0.05).

The concentration of nickel found ($n = 6$), $2.40 \times 10^{-7} \pm 4.15 \times 10^{-8}$ mol dm^{-3} ($k = 2$), was in good agreement with the value obtained ($n = 3$), $3.06 \times 10^{-7} \pm 1.97 \times 10^{-8}$ mol dm^{-3} ($k = 2$), when using hydride generation atomic absorption spectrometry as a reference technique.

It is worth pointing out that in the calculation of combined uncertainty (u_T), the day-to-day variability was included (Tables 6 and 7). A detailed analysis of the sources of uncertainty highlights the fact that the great uncertainty was due to the voltammetric technique used when the samples were UV irradiated.

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