Spectrophotometric Flow Injection Determination of Lead in Port Wine Using In-line Ion-exchange Concentration

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A flow injection system with in-line ion exchange is proposed for the spectrophotometric determination of lead in Port wine. A Chelex-100 resin mini-column (200-400 mesh) is used for lead concentration, and the chromogenic reaction is based on the formation of a ternary complex between lead, Malachite Green and iodide. Forty-five digested wine samples can be run per hour (25-500 \textmu g l\textsuperscript{-1} Pb) and results are comparable to those obtained by ETAAS. Other features are the measurement precision (RSDs lower than 2.6\%), a detection limit of 12 \textmu g l\textsuperscript{-1} and a sampling rate of 45 h\textsuperscript{-1}.

Keywords: Flow injection analysis; lead determination; Port wine: spectrophotometry; ion exchange

Introduction
There has been growing interest in the control of the heavy metal content in wines owing to the public health aspects involved. Among the heavy metals usually present in wines, lead is the most important toxicologically.\textsuperscript{1} Winery equipment, lead-containing pesticides and environmental pollution, such as car exhausts, are considered to be the major sources of contamination. These concerns led to the establishment of maximum permissible lead content levels; in Europe, the Office International de la Vigne et du Vin (OIV)\textsuperscript{2} set a maximum limit of 0.3 mg l\textsuperscript{-1} and the USA and Canada set maximum values of 0.1 and 0.2 mg l\textsuperscript{-1}, respectively.\textsuperscript{2}

The OIV reference procedure\textsuperscript{2} for the determination of lead in wines is ETAAS. Some modifications of this protocol have been suggested to meet the specificity of some wines such as Port.\textsuperscript{2} Other procedures involving flame AAS or spectrophotometric methods with previous sample digestion have also been reported.\textsuperscript{3}

The main purpose of this work was to develop an alternative procedure for lead determination in Port wine, using a flow injection system with spectrophotometric detection. The method was based on a previously described reaction for the determination of cadmium,\textsuperscript{3} in which lead was an interfering agent. The reaction involved the formation of a ternary complex between Malachite Green (MG), iodide and the metal cation. The flow injection manifold included a Chelex-100 mini-column to concentrate lead from the digested wine samples.

Experimental
Reagents and Solutions
All solutions were prepared with de-ionized water and analytical reagent-grade chemicals.

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mesh, sodium form). The resin was dispersed in the conditioning buffer and further introduced into the column by means of a syringe. Ordinary dishwashing foam was placed at both ends of the mini-column to entrap the resin, which was thereafter conditioned in the flow injection system by consecutive injections of 0.05 mol L⁻¹ HNO₃ solution.

The determinations related to the reference method were carried out in a Perkin-Elmer (Norwalk, CT, USA) Model 4100 ZL atomic absorption spectrometer with electrothermal atomization, using the operating conditions described elsewhere.³

Procedure

The manifold for studying the chromogenic reaction is presented in Fig. 1. The standards (S) were introduced into a carrier stream (C) with the same acidity (0.05 mol L⁻¹ HNO₃) as the digested wines. The injected plug was mixed with a buffer solution (R₁) at a concentration in order to achieve a pH value in the range 3.5-4. Subsequently, the dispersed zone merged with the reagents MG and KI at confluence b, and the chromogenic reaction started inside reactor B₁, which was immersed in an ice-bath. Thereafter, the processed plug passed through the flow cell and the transient absorbance was recorded as a peak with height proportional to the lead content in the injectate.

The influence of the water-bath temperature (40°C), pH (2.0-4.5) for reaction development concentrations of KI (1.6-4.0 mol L⁻¹) and MG (1.0 x 10⁻³-1.0 x 10⁻⁴ mol L⁻¹) and reactor lengths (3-200 cm) on the analytical signal were assessed by using a 500 µL sampling loop (L₁) and fixed flow rates (Fig. 1). Variations in pH were attained by using acetic acid-acetate buffer solutions with different concentration ratios.

The flow injection system incorporating the chelating ion exchange resin is shown in Fig. 2.

In the concentration position, the sample plug was carried by 0.05 mol L⁻¹ HNO₃ solution (C), merged at confluence d with the conditioning buffer (R₂) and flowed through the column, where lead was retained and concentrated. The in-line concentration step was time controlled. When the commutator was switched, the eluent (0.5 mol L⁻¹ HNO₃) passed through the column and washed out the lead ions. The resulting plug was mixed at confluence a with R₄ solution to adjust the pH to 3.5-4 and to suppress copper interference (picolinic acid). Potassium iodide and MG were then added at confluence b and the plug was processed similarly as mentioned above. This system (Fig. 2) was optimized in relation to the preconcentration pH and concentration time and as the eluent concentration. A detailed re-evaluation of the parameters related to the chromogenic reaction (lengths of B₄ and flow rates of the reagents MG and KI) was performed, to adjust them to the changes introduced by the preconcentration step.

After system optimization, the main analytical characteristics were evaluated: detection limit, precision and sampling rate. Thereafter, standards and samples (wine digestes), already analysed by ETAAS, were injected in duplicate into the flow system and the concentrations were calculated from the calibration graph.

Results and Discussion

A flow injection system was first developed and optimized in order to maximize the sensitivity (slope of the calibration curve) and obtain a low detection limit. The concept of this assembly was based on a previous one developed for the determination of cadmium using the same reaction.³ However, the detection limit of this system was not low enough for lead determination in Port wines (expected concentrations below 200 µg L⁻¹), and so a preconcentration step was introduced into the system to allow the determination of lower concentration levels.

Optimization of the Flow Injection System Without Ion Exchange

The system is very dependent on temperature variations. Within the tested temperature range (4-40°C) the signal increased as the temperature decreased (Fig. 3). The temperature was set to about 4°C as this corresponds to the temperature obtained with an ice-bath.

Regarding the acidity inside reactor B₁ (Fig. 1), a pH in the range 3.5-4 was used. For a 500 µg L⁻¹ lead standard, the absorbance increased 3.3-fold with increase from pH 2.0 to 3.0, remained approximately constant up to pH 4.2 and then decreased 1.2-fold with an increase to pH 4.5.

On increasing the KI concentration from 1.0 to 4.0 mol L⁻¹, the signal increased up to 2.0 mol L⁻¹, with a further slight increase being observed for higher KI concentrations. Moreover, at iodide concentrations ≥2.0 mol L⁻¹ precipitation effects were observed when MG concentrations ≥7.5 x 10⁻⁴ mol L⁻¹ were used. The concentration of KI was then selected as 2.0 mol L⁻¹.

![Fig. 1. Flow injection system for the study of the chromogenic reaction.](image)

![Fig. 2. Flow injection system with in-line ion exchange concentration for the determination of lead in Port wines.](image)
mol l⁻¹. The MG concentration was set at 5.0 × 10⁻⁴ mol l⁻¹, since above this value an increase in the baseline noise was observed, probably owing to the precipitation of MG on the flow cell and tubing walls.

The length of reactor B₂ should be as small as possible to minimize dispersion of the injected plug, yet sufficient to allow the mixing of the plug with the buffer stream; it was set as 30 cm. The length of B₂ should not be selected too short, since its main function was to provide a given time interval for KI-MG interaction prior to their addition to the sample. It was varied from 3 to 100 cm and a value of 50 cm was chosen as this corresponded to the maximum sensitivity. The decrease in sensitivity observed with longer tubes was probably due to precipitation effects as a consequence of micellar growth inside the reactor. The length of B₂ was selected as 150 cm as the best compromise between sampling rate, sensitivity, possibility of precipitation and measurement precision.

System With the Ion-exchange Mini-column

In the flow system incorporating the chelating ion exchange resin (Fig. 2), for a fixed flow rate and loop (L₂) volume, the amount of lead flowing through the resin was controlled by the time interval between commutations required for sample injection and elution (also leading position). The signal underwent a 200% increase when this interval was changed from 15 to 30 s, and only 7% from 30 to 90 s. Therefore, a concentration time of 30 s was set.

Regarding the three pH values tested for concentration (4.3, 8.5, 9.5), maximum sensitivity was observed at pH 4.3, which confirmed details supplied by the manufacturer.

The eluent concentration was investigated in the range 0.25–1.0 mol l⁻¹ HNO₃. A concentration of 0.5 mol l⁻¹ was adopted as there was no significant improvement in signal intensity and return to baseline (elution efficiency) at higher concentrations. Moreover, the use of higher acid concentrations would make it more difficult to buffer the resulting solution. In the system in Fig. 2, the length of reactor B₂ was set as 200 cm. Maximum sensitivity was attained with this length, which provided a narrow baseline (<0.002 A).

When the flow rates of the MG and KI streams were increased from 0.4 to 0.8 ml min⁻¹, a systematic increase in the signal was observed, but beyond 0.7 ml min⁻¹ the baseline became unstable. The flow rates of the two reagents were therefore set to this value.

Interference from copper was observed as it also forms a ternary complex with MG and KI. In order to overcome this interference, picolineate, which forms a very stable, colourless complex with copper, was included in solution R₄. A picolineate acid concentration of 1 × 10⁻² mol l⁻¹ was used and copper concentrations as high as 3 mg l⁻¹ were suppressed. This is sufficient as the maximum copper content in wines is not expected to exceed 1 mg l⁻¹. The interference of cadmium was not studied as its content in wines is usually substantially lower than that of lead. In addition, at the preconcentration pH used, lead retention in the ion-exchange resin is much higher than that of cadmium.

Application to Wine Analysis

In order to assess the quality of the results obtained with the developed flow injection system for the determination of lead in Port wines, 20 digested samples were analysed by the proposed procedure and by ETAAS. The values obtained and their differences are presented in Table 1. From a linear regression between the results obtained with the two methods, the 95% confidence limits obtained for 18 degrees of freedom (t-value = 2.10) were 0.81 ± 10.29 μg l⁻¹ for the intercept and 1.02 ± 0.080 for the slope; these results indicate good agreement between the two methods. The results obtained by the proposed procedure were precise, with RSDs (n = 5) of 2.6, 2.5 and 1.2% for wines with concentrations of 35, 50 and 80 μg l⁻¹, respectively. The detection limit was determined as 12 μg l⁻¹ according to IUPAC recommendations. The sampling rate achieved was about 45 determinations per hour.

Conclusions

The flow injection system with spectrophotometric detection proposed for lead determination in Port wine is an alternative to more expensive methodologies such as AAS. It provides good-quality results, in terms of accuracy and precision (RSDs lower than 2.6%), and allows 45 determinations per hour for the digested samples.

With some adaptations, the described methodology can be applied to the determination of low levels of lead in other matrices.

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**Table 1 Comparison between the results obtained by FI and by ETAAS**

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<th>ETAAS</th>
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**Fig. 3 Influence of temperature on the analytical signal (A) for a lead concentration of 500 μg l⁻¹, obtained with the system in Fig. 1.**
for critical comments. The collaboration of Cocioburn Smithes
(Gaia, Portugal) is also acknowledged.

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