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ORIGINAL PAPER

A new method for the simultaneous determination of Fe(III), Cu(II), Pb(II), Zn(II), Cd(II), and Ni(II) in wine using differential pulse polarography

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Abstract A new and simple differential pulse polarographic method for the analysis of wine has been established. With this method, it was possible to determine simultaneously six trace elements in wine. There was no need for time consuming extraction and separation procedures with danger of contamination. The polarogram of wet digested wine was taken initially in pH 2 acetate buffer and Pb, Cd, and Zn were determined by standard additions. Ethylene diamine tetraacetic acid (EDTA) was added and pH was increased to six by addition of NaOH. Fe and Cu were determined subsequently. The ammonia buffer, pH 9.5, was identified as the best medium for separation and determination of Ni and Zn. The quantities of trace elements were found as Cu 290 ± 20 μg L^{-1}, Fe 8960 ± 50 μg L^{-1}, Pb 148 ± 17 μg L^{-1}, Cd 16 ± 8 μg L^{-1}, Zn 460 ± 25 μg L^{-1}, and Ni 78 ± 17 μg L^{-1}.

Keywords Wine · Analysis · Determination · Trace elements · Polarography

1 Introduction

Wine is a widely consumed beverage all over the world. This is due in part to the convenience of process for manufacturing. The ease of processing wine, as it does not require a fancy apparatus, enables manufacturing even in private houses.

Composition of wine is influenced by many factors including, grape varieties, soil chemistry, atmospheric precipitations, pesticides, and materials used during production, transport, and storage.

The consumption of wine in moderate quantities may partially supply human nutritional requirements for essential elements such as K, Ca, Mg, Cr, Co, Fe, F, I, Cu, Mn, Mo, Ni, Se, and Zn [1, 2].

On the other hand, several metals, such as Pb, As, and Cd are known to be potentially toxic [3]. With the possibility of accumulating in bones, lead may act as a metabolic poison, replacing calcium. Cadmium, on the other hand, may accumulate in liver and kidney. Sometimes the daily intake of these heavy metals may increase above the permissible levels. Thus, in case of excessive intake, the analysis for certain elements in wine is of special interest.

Higher precision instruments have been used in previous studies for trace element analysis in wine [4–6]. Most application of inductively coupled plasma mass spectroscopy (ICP-MS) in wine analysis has been devoted to the determination of rare-earth elements; there has been little study of multi-element composition. Considering wine as a complex matrix with many organic and inorganic substances that can affect signal intensity in ICP-MS measurements, these kinds of matrix effects and plasma instability related to the ethanol content were minimized by 1:1 dilution with HNO_3 [4, 7] or alcohol was removed by evaporation to dryness and the residue was dissolved in nitric acid [8]. However, according to one work [6], partial loss of Ni and Zn was found after microwave digestion and high pressure ashing. In that study [6], both ICP-MS and total reflection X-ray fluorescence (TXRF) was used for comparison and agreement better than ±50% was found between these two techniques for Fe, Cu, Zn and Pb. However, for Ni it was more than 200%, although a direct measurement without digestion was used.

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The content of some metals in wine has been determined by several techniques. Among these, most commonly used were flame atomic absorption spectrometry (FAAS) [9, 10], electrothermal atomic absorption spectrometry (ETAAS), [11, 12], ICP-MS, and [13, 14] inductively coupled plasma optical emission spectrometry (ICP-OES). By using ETAAS, Cu, Zn, and Fe were determined in wine samples [11]; Cd and Pb were determined in one other work using the same method [12]. ICP-OES method has been used for Pb determination in wine [13] and for the determination of variations in the metal distribution in wine of one region [14]. Synchrotron radiation total reflection X-ray fluorescence spectrometry (SRTXRF) method has been used to classify, to characterize, and to determine the elements in wine [15].

For the direct determination of Cd as a toxic metal in wine, ETAAS has been used because of insufficient detection capacity of ICP-OES; other elements such as Zn, Fe, Ni, Cr, Cu, and Pb were determined with ultrasonic nebulizer-inductively coupled plasma optical emission spectrometry (USN-ICP-OES) methods [16]. Several methods have been developed for lead determination as a toxic element in wine. It was shown that wine contains chelates with high capacity for lead complexation [17]. Because of these chelating agents, wine sample has to be digested first with HNO_3 and H_2O_2 before application of the method [9].

There are very few data in the literature where electroanalytical methods have been applied for wine analysis. Differential pulse adsorptive stripping voltammetry has been used [18] for the determination of nickel in wine. Digestion of the sample was made by using H_2SO_4 and H_2O_2. Dimethylglyoxime was used as the complexing agent and it was found that the Ni concentration in wine was 1.05×10^{-7} M. Lead in wine was determined by ASV [19] and the effect of different pretreatment procedures has been studied. Copper, lead and zinc were determined by differential pulse polarography [20] in different electrolytes than we used.

The reason that electrochemical methods have rarely been used in wine analysis maybe the need of good knowledge in electrochemistry and also difficulty in automation. However, the high sensitivity and reproducibility of these techniques, combined with inexpensive instrumentation compared with spectroscopic methods, make them eminently suited for this task.

In this article, the determination of many trace elements in wine by differential pulse polarography (DPP) has been reported. This method was used first time for wine analysis. There is no need of separation and enrichment procedures which are time consuming and have risk of contamination.

2 Experimental

2.1 Apparatus

A PAR Model 174A polarographic analyzer system, equipped with a PAR mercury drop timer, was used. The natural drop time of the electrode was in the range 2–3 s (2.35 mg s^{-1}). A Kalousek electrolytic cell with a saturated calomel electrode (SCE), separated by a liquid junction, was used in the three-electrode configuration. The counter electrode was platinum wire. The polarograms were recorded with a Linseis (LY 1600) X–Y recorder under the conditions of a drop life of 1 s, a scan rate of 2 or 5 mV s^{-1}, and pulse amplitude of 50 mV.

2.2 Reagents

All chemicals used were of analytical reagent grade and triply distilled water was used in preparation of all solutions. $FeCl_3 \cdot 6H_2O$, $Cu(NO_3)_2 \cdot 3H_2O$, $Zn(NO_3)_2 \cdot Pb(NO_3)$, $3CdSO_4 \cdot 8H_2O$, $Ni(NO_3)_2 \cdot 6\ H_2O$, and sodium salt of EDTA salts have been used for the preparation of standard solutions.

Nitric acid (65%), perchloric acid (70%), and hydrochloric acid (37%) were used in digestion procedure. The mercury (Analar) used in the dropping mercury electrode was obtained from BDH Chemicals Ltd., Poole, England. The 0.1 M stock solutions were used for the preparation of working solutions of 10^{-3} M by daily dilution to prevent the solution from ageing. Contaminated mercury was cleaned by passing it successively through dilute HNO_3 (3.0 M) and water columns in the form of fine droplets using a fine platinum sieve. The collected mercury was dried between sheets of filter paper. A polarogram of this mercury was taken before use to ensure the absence of impurities. The same digestion period was applied to the same acid mixture without the addition of trace elements and the polarogram was taken under the same conditions, no peak for impurity was observed.

2.3 Digestion of samples

According to the literature [9, 18], in all given methods, wine has to be filtered and then digested. In this work, we used wet digestion procedure with acids. For this purpose, 500 mL of wine (ordinary quality) was evaporated first into 15 mL. Then it was wet digested in a long- necked (30 cm) 100 mL flask. Its mouth was covered with a glass funnel. The total acid used was 67 mL of $HClO_4$ and 25 mL of HNO_3. Nitric acid was added when the color of the solution became dark brown to protect it from explosion. Digestion was completed with the appearance of white fumes of

perchloric acid when approximately 1.0 mL solution remained. The digested sample which was clear and colorless was cooled to room temperature, rinsed the funnel into flask with water, and the contents were transferred into 10.0 mL calibrated flask, making up to mark with triply distilled water. This sample was kept in a Teflon bottle in refrigerator. The same amount of acids, after vaporization had no impurity peak when polarogram was taken under same conditions. Since one of the main points in this work was to determine lead, H_2SO_4 was not used because of danger of $PbSO_4$ formation [21].

2.4 Polarographic determinations

A total of 10 mL electrolyte was de-aerated by a stream of nitrogen gas (99.999%) for about 10 min. Polarograms were taken by scanning the potential in the negative direction from 0.0 V to −1.5 V depending on pH, at a scan rate of 2 or 5 mV s^{-1}. The peak potentials of Fe, Cu, Zn, Cd, Ni, and Pb which are commonly found in foodstuffs were determined at different electrolytes in the presence of digested wine sample. The polarogram of the digested sample was taken under various conditions and the trace elements in the sample were determined by standard additions.

2.5 Procedure

According to our preliminary investigations mentioned in "Results and discussions" section, we found that the below given procedure has to be applied for the determination of elements in wine.

A spike of digested sample is added into the polarographic cell containing pH 2 acetate buffer. Pb, Cd, and Zn are determined by standard additions, then EDTA is added, pH is increased from 5 to 6 with NaOH, and Fe and Cu are determined. For separation and determination of Ni and Zn, the best medium is found to be as ammonia buffer pH 9.5; they are determined in this medium.

3 Results and discussions

The peak potentials of elements are strongly dependent on the nature of the medium. Although the peak potentials are known approximately in certain electrolytes, they may change in the presence of digested sample because of interference and complex formation [22, 23]. Having established the peak potentials at different pH values, it is then possible to tell from a polarogram which elements are likely to be present in the sample. The use of one polarogram, however, may be misleading, because at that particular pH the peaks may have overlapped. Therefore, verification by taking a second polarogram at a different pH is advisable. This procedure has the additional advantage that the presence of peaks in two polarograms provides a more accurate determination of the actual amount of an element.

Polarograms of the digested wine (İkram, medium quality) samples were taken under several pH and buffer conditions. It was possible separating some overlapping peaks by the addition of EDTA or ammonia using their complexing ability. At each condition, the elements commonly found in food samples were added into the polarographic cell and their peak potentials were recorded.

In a polarogram taken at pH 2 acetate buffer, there were peaks at −0.4 V, −0.6 V (very small), and at about −0.95 V. According to our preliminary studies, they may belong to lead, cadmium, and zinc, respectively. Their presence was confirmed by standard additions and also by taking polarograms at different conditions.

3.1 Determination of copper and iron

Copper may enter into wine from copper-based vineyard sprays. Iron on the other hand is found in all grape varieties in substantial quantities, and thus is found in wine in remarkable quantities. For their polarographic determination, various electrolytes have been used. The reduction of copper and iron takes place near 0 V at low pH values, and thus they cannot be separated in this medium. Their reduction peaks can shift to more negative potentials in the presence of a complexing agent such as EDTA. We found that iron and copper peaks can be separated in EDTA at pH values higher than 4, but at lower pH values they overlap. A polarogram of wine sample, Fig. 1, in EDTA at pH 6 had three peaks at about −0.18 V, −0.4 V, and −1.25 V which belong to the EDTA complexes. The first peak at −0.18 V belongs to iron, the second peak at −0.40 V to copper, and the third peak at −1.25 V to zinc; their quantities were determined with standard additions. Polarograms for copper determination are given in Fig. 1, and for iron determination in Fig. 2. Since iron content was much larger than copper, it was not possible to determine it on the same sensitivity conditions used for copper. As can be seen from Fig. 2, 10^{-2} M Fe(III) (10^{-4} M in cell) additions had to be made instead of 10^{-3} M which is used commonly, because of very low sensitivity conditions. Thus, while lower sensitivities could be used for iron, higher sensitivities have to be used for copper because of its low concentration. Copper content found was 305 ± 25 μg L^{-1} in this medium. According to Office International de la Vigne et du Vin (OIV), the acceptable limit of copper is 1 mg/L.

For validation of this result, the copper content was determined also in ammonia buffer at pH 9.5. Cu(II) has a peak at −0.4 V in this medium, and the quantity found was

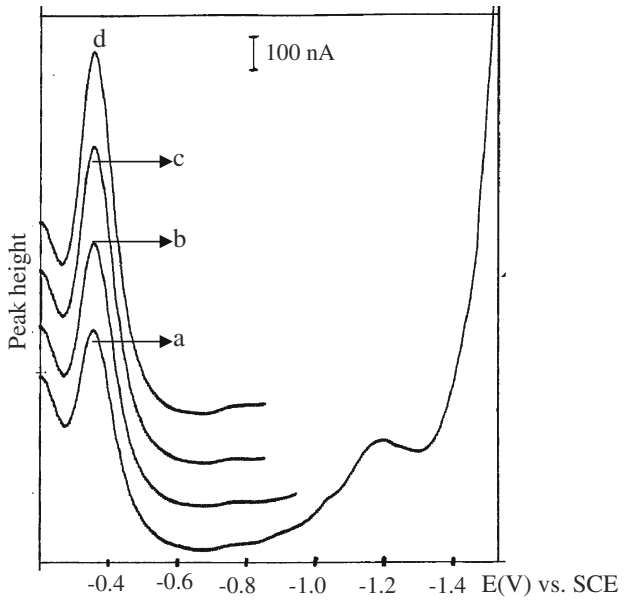

Fig. 1 Differential pulse polarographic determination of copper in red wine sample (**a**) 9 mL HAc/Ac^- buffer + 0.5 mL 0.3 M EDTA + 2 mL sample (pH = 6); (**b**) a + 0.1 mL 10^{-3} M Cu(II); (**c**) b + 0.1 mL 10^{-3} M Cu(II); (**d**) c + 0.1 mL 10^{-3} M Cu(II)

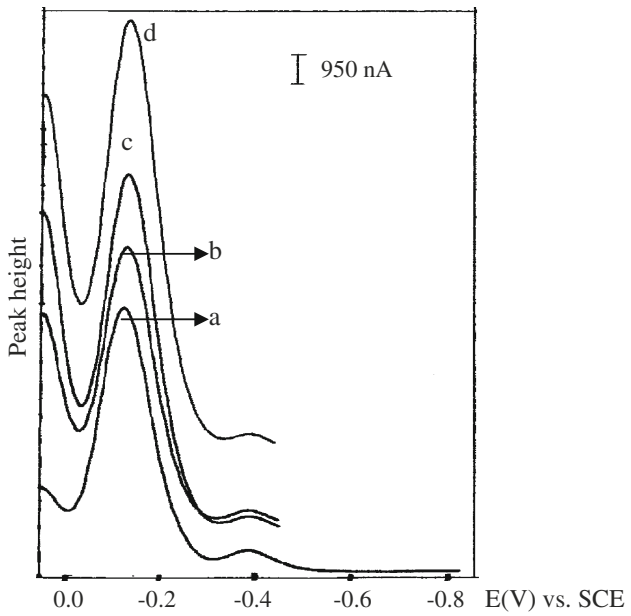

Fig. 2 Differential pulse polarographic determination of iron in red wine sample (**a**) 9 mL HAc/Ac^- buffer + 0.5 mL 0.3 M EDTA + 1.0 mL sample (pH = 6); (**b**) a + 0.5 mL 10^{-3} M Fe(III); (**c**) b + 0.2 mL 10^{-2} M Fe(III); (**d**) c + 0.2 mL 10^{-2} M Fe(III)

very similar to the previously obtained result. The average copper content for two conditions was 290 ± 20 μg L^{-1}.

The iron content found was 8,960 ± 90 μg L^{-1} which is quite high, but as will be seen, it is high in various kinds of wines also. The normal concentration of iron, according to OIV, in wine may change between 2 and 10 mg/L. The obtained results are summarized in Table 1, and for comparison, quantities found for different wine varieties from several countries have been added. In this table, the results given for Canadian wines (second column) are from Okanagan Valley and Niagara Peninsula [4]. The elemental concentration ranges for Brazilian wines [15] are given in the fifth column of Table 1. When compared, it can be seen that the iron and copper quantities are more or less in the same level for various wines, except for the last column where copper and zinc quantities are much larger [6] than the others. In this work [6], the effect of digestion methods has been investigated.

3.2 Determination of lead, cadmium, and zinc

Cadmium and lead are known as potentially toxic elements for human body. Cadmium levels in wine can be due to residues of agrochemical products and from industrial complexes near to vineyards. Also cadmium may be due to contacts with the apparatus used in wine production. Usually, the cadmium content in wine is quite low, but when large quantities are consumed, then it may reach the maximum allowable levels.

Wine is one of the most highly consumed alcoholic beverages worldwide, but has the disadvantage of containing the highest level of lead compared to others. Thus, apparently attention is paid to establish new methods for its determination**.** The presence of lead in wine is due to natural source of contamination associated to ground and also related to atmospheric precipitation, pesticides, and materials used in production, transport, and storage of the wines.

These elements in wine were determined at pH 2 acetate buffer from their peaks at −0.4 V for lead, at −0.6 V for cadmium, and at −1.0 V for zinc. As can be seen from Fig. 3, cadmium peak was very small, but zinc and lead peaks were large and could be determined precisely. By standard addition, the lead content was found to be as 150 ± 17 μg L^{-1}, cadmium 16 ± 8 μg L^{-1}, and zinc 470 ± 25 μg L^{-1} (Fig. 4)**.** For the determination of lead, care has to be taken during digestion; it is better not to use H_2SO_4 to protect from $PbSO_4$ formation. Since HCl had to be used for the reduction of Se (VI), the formation of $PbCl_2$ was also possible**.** For this purpose, before the sample aliquot was taken, it was warmed up so that possible precipitate of $PbCl_2$ could be dissolved. However, no difference in peaks and peak heights were observed indicating that no precipitation had taken place.

Lead level found in this wine, was lower than the upper limit established by OIV, which is at present (2007) 150 μg/L. Acceptable limits (OIV) were 10 μg/L for Cd and 5 mg/L for Zn.

Table 1 Trace elements in various red wines (μg L^{-1})

Ions	This work	Canadian wine [4]		Argentine wine [16]	Brazil wine [15]	Various wines [6]
		Okanagan valley	Niagara peninsula			
Cu	290 ± 20	55	133	27	400	3,000–6,000
Fe	8960 ± 90	1,080	2,500	650	1,700–5,200	7,000–9,600
Ni	78 ± 12	21	25	–	20–300	60; 140
Cd	16 ± 8	0.47	0.67	3.6	–	111; 3.8
Pb	150 ± 17	12	28	–	–	208–440
Zn	470 ± 25	468	787	110	200–1,300	6,300–11,200

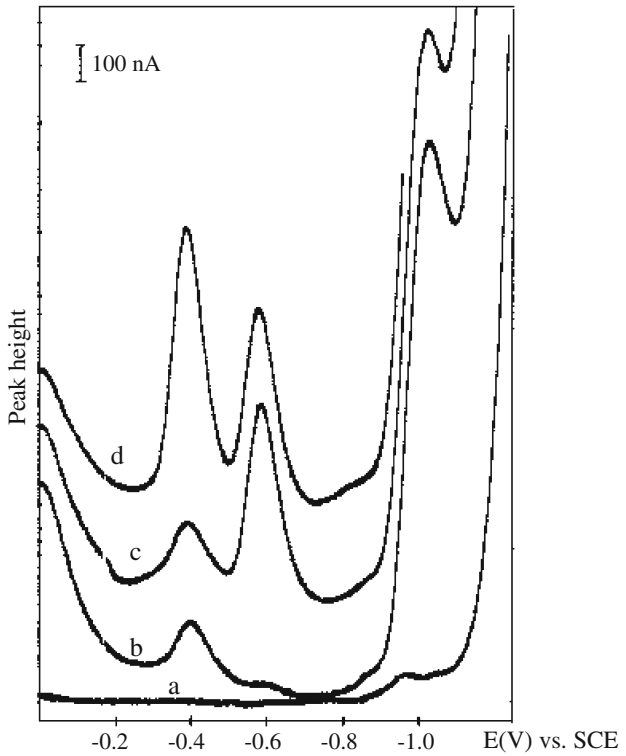

Fig. 3 Differential pulse polarographic determination of cadmium and lead in red wine sample (**a**) 9.0 mL HAc/Ac^- (pH = 2); (**b**) a + 1.0 mL sample; (**c**) b + 0.1 mL 10^{-3} M Cd(II); (**d**) c + 0.1 mL 10^{-3} M Pb(II)

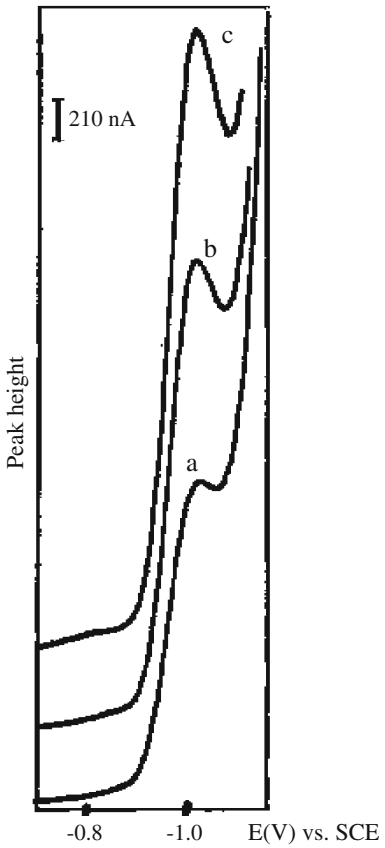

Fig. 4 Differential pulse polarographic determination of zinc in red wine sample (**a**) 9 mL HAc/Ac^- (pH = 2) + 1 mL sample; (**b**) a + 0.2 mL 10^{-3} M Zn(II); (**c**) b + 0.2 mL 10^{-3} M Zn(II)

3.3 Determination of zinc and nickel

Zinc can be carried over into wine from the plants since they absorb zinc from soil in small quantities. The zinc content may also increase, when zinc containers are used during the processing. Nickel is present in wines owing to the use of nickel containing stainless steel containers for wine fermentation and storage in modern cellar technology. There are only a few data in the literature [15, 18] for the determination of nickel in wine. The nickel content in Brazil wine [15] was changing between 20 and 300 μg L^{-1}.

According to our preliminary studies, zinc and nickel could be well separated, due to their ammonia complexes, Fig. 5, in ammonia buffer at pH 9.5. In this medium, nickel peaks were at −1.0 V and −1.5 V and zinc peak was at −1.30 V**.** Their quantities were calculated by standard additions as 78 ± 12 μg L^{-1} for Ni (from the peak at −1.0 V) and 440 ± 30 μg L^{-1} for zinc. As can be the seen, quantities for zinc, found in two different media, pH 2 acetate buffer and pH 9.5 ammonia buffer were very similar.

3.4 Application to white wine

A very good quality of white wine (Antik Doluca) is taken and similar digestion procedure is applied. For 500 ml of

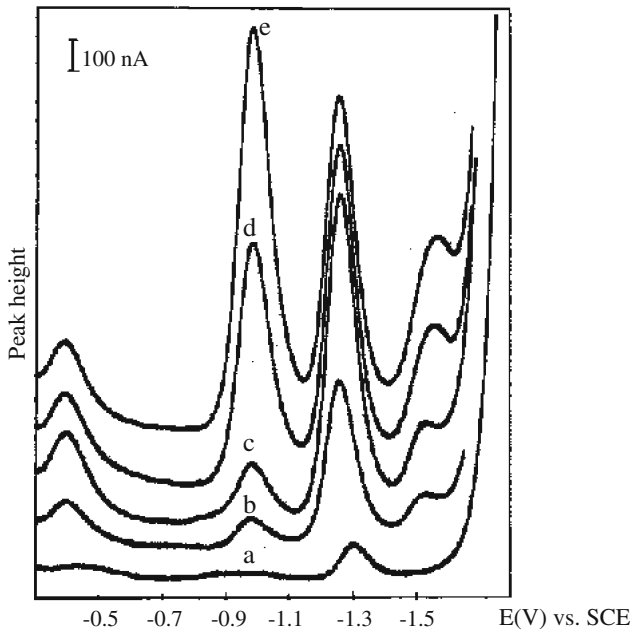

Fig. 5 Differential pulse polarographic determination of nickel in red wine sample (**a**) 9.5 mL NH_3/NH_4Cl (pH = 9.5); (**b**) a + 0.5 mL sample, Current Range = 0.2 mA; (**c**) Current Range = 0.1 mA; (**d**) c + 0.1 mL 10^{-3} M Ni; (**e**) d + 0.1 mL 10^{-3} M Ni

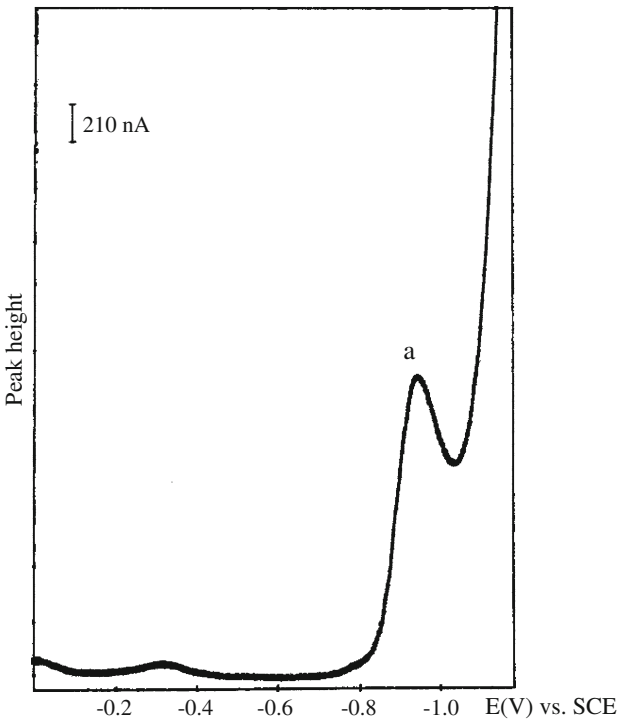

Fig. 6 Differential pulse polarogram of white wine sample (**a**) 9 mL HAc/Ac^- (pH = 2) + 1.5 mL sample

wine 75 ml $HClO_4$ and 25 ml HNO_3 had to be used. A polarogram of white wine obtained at pH 2 is given in Fig. 6. As can be seen, only zinc peak is large enough to observe, while the others are quite small. Trace element quantities were determined with the same procedure as given above for red wine. Results obtained are summarized in Table 2 with some literature values for white and red wines for comparison. As can be seen, iron content, 598 ± 30 μg L^{-1}, is much smaller than 8,960 ± 90 μg L^{-1} obtained for red wine. This is due to the difference in grapes varieties. Same kind of difference was also observed for Brazilian wines [15], while iron content was changing between 800 and 2,400 μg L^{-1}, for white wine, it was between 1,700 and 5,200 μg L^{-1} for red wine. The lead content for white wine was only 24 μg L^{-1} much lower than 148 μg L^{-1} for red wine, an indication of its high quality.

For validation, a synthetic sample containing similar quantities of elements and ethyl alcohol has been digested as given above, and then the trace elements present have been determined. It was found that the above-proposed method could safely be used for the determination of elements in wine.

4 Conclusions

The present investigation has shown that the trace elements in wine can be determined simultaneously by using DPP. For this purpose, the digested sample was first added into a solution in the polarographic cell at pH 2. Pb, Cd, and Zn were determined from the polarogram by standard

Table 2 Trace elements in various red and white wines (μg L^{-1})

Ions	This work		Argentine wines [16]		Brazil, Portugal, Chile [15]	
	White wine "Antik Doluca"	Red wine "İkram"	White wine	Red wine	White wine	Red wine
Fe	598	8960	600	650	800–2,400	1,700–5,200
Ni	15	78	ND	ND	120	20–300
Cu	46	290	26	27	100	400
Zn	337	470	95	110	300–600	200–1,300
Cd	–	16	1.2	3.6	–	–
Pb	24	150	60	85	–	–

additions. Then the pH was increased to 6 and EDTA was added, and Fe and Cu were determined. This implied that by using one solution in cell, five elements could be determined simultaneously only by changing the pH. For Ni on the other hand, the medium had to be ammonia buffer, in order to separate Zn and Ni, since in the above given supporting electrolytes, zinc and nickel peaks were not well separated.

This study also verified that if determination of one ion was doubtful, a second polarogram might be taken at a different pH, thus providing a means for a new determination and comparison with the initial value.

This method seems to enable simultaneous determination of trace elements in wine by using an inexpensive instrument and without any time consuming separation or pre-concentration procedures.

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