Study of Lability of Heavy Metals in Wines with Different Degrees of Aging through Differential Pulse Anodic Stripping Voltammetry

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DPASV is a precise, highly sensitive, low-cost analytical technique which allows for the determination of total content of trace metals and at the same time provides information on the stability of metal-ligand unions. In this study, the content of Zn, Cd, Pb, and Cu in three wines with different aging periods subjected to three different treatments (digestion, acidification to pH 1, and dilution) was studied to determine total metal concentration and distinguish the labile fraction from that displaceable by acids. Previously, the influence of deposition time and sample volume on potential and intensity of each metal was studied. Zn is the cation with the greatest total content and lability. Pb is complexed to the natural pH of wine but is easily displaceable at pH 1. Cu forms very strong unions at pH 1, and stability is even greater if the wine is young and organic matter is not very oxidized. Concentration of Cd is approximately 1 $\mu g/L$.

INTRODUCTION

Differential pulse anodic stripping voltammetry (DPASV) is an accurate, extremely sensitive, low-cost technique. This makes it more advantageous than other methods used in solving various analytical problems. In fact, the use of this method is becoming increasingly widespread in the determination of heavy metal content in samples such as wine, milk, and water. Its optimal sensitivity levels are due, to a great extent, to the initial preconcentration that metallic ions undergo on the drop or on the mercury film, which acts as a working electrode (Bond, 1980; Riley and Watson, 1987; Bersier, 1987; Willard et al., 1988).

Compared to conventional techniques such as atomic absorption spectrophotometry (AAS), this technique provides information on the stability of unions between the metal and organic and inorganic ligands present in the sample. Not only can the total metal content be determined but also the labile metal content. Labile metal comprises free metal ion and metal that can be dissociated from complexes or colloidal particles and, hence, deposited in the mercury electrode without modifying the initial medium, so as not to perturb the equilibria in which they participate. With DPASV, a complex is operationally defined as "labile" if it provides an identical signal to that from an equal concentration of free metal (Buffle, 1988). The labile fraction presents, in general, the greatest toxicological risk because it is more easily absorbed by organisms (Florence, 1989).

Qualitative determination of the element is carried out through peak potential, E_p, while quantification is obtained from peak height, i_p, which, under ideal experimental conditions, is proportional to concentration.

In theory, the highest sensitivity should be obtained with sufficient deposition times, so that all the metal is deposited in the electrode. However, it is better to avoid long deposition times because they cause proportionality loss problems between the signal and metal concentration due to, among other causes, the formation of intermetallic compounds (Daniele et al., 1989; Psaroudakis and Efstathiou, 1987) and the diffusion of the metal into mercury (Bond, 1980). It is sufficient to develop this step under repeatable conditions. In this way, with small deposition times, time savings are achieved with only a moderate loss in sensitivity (Willard et al., 1988). In general—and

without needing too many previous checks—deposition times that reduce metal concentration in matrix by 2% are used. This allows the nature of the solution to remain essentially unaltered, a prerequisite for quantification through standard addition (Riley and Watson, 1987).

The presence of organic matter interferes with measurements through adsorption on the electrode or formation of organometallic compounds (Sagberg and Lund, 1982; Chau and Lum-Shue-Chan, 1974; Gregor and Powell, 1988) which alter both peak current, i_p, and potential, E_p. Organic matter adsorption results in a decrease of i_p values and a shift in E_p toward more positive values, producing wider peaks (Sagberg and Lund, 1982). Wine, a substrate in this study, is a complex aqueous matrix consisting of a large number of organic compounds so this type of interference can therefore be expected (Marin and Ostapczuk, 1992).

The aim of this study was to compare the influence of deposition time, t_d, and sample volume, V_s, on, i_p and E_p values for three red wines with different aging, from Navarra (Spain). Every wine underwent three different treatments (wet digestion with H_2SO_4/H_2O_2; acidification to pH 1 with HCl; and dilution with deionized water) to observe the effect of the susbtrate on Zn, Cd, Pb, and Cu and to analyze total, acid free, and labile concentrations. Acid digestion allows the quantification of total metal content as well as the observation of interferences between metals and those caused by the acid matrix. Acidified samples (pH 1) shift metal balances toward free metal, and in the case of samples diluted with distilled water, natural balance is barely perturbed, thus yielding labile metal.

MATERIALS AND METHODS

Materials. The samples were three commercial red wines from the same wine cellar of Navarra Denomination of Origin (NDO), elaborated with *Vitis vinifera Garnacha*. The wines' characteristics are shown in Table I.

Reagents used were Merck analytical quality with controlled heavy metal content.

The metal standard solutions (1000 mg/L) were obtained from Merck. Solutions of lower concentrations were prepared by dilution of the stock solution immediately before use.

Borosilicated glass material was washed with deionized distilled water, cleaned for 24 h with 10% nitric acid, and, finally, rinsed several times with deionized distilled water.

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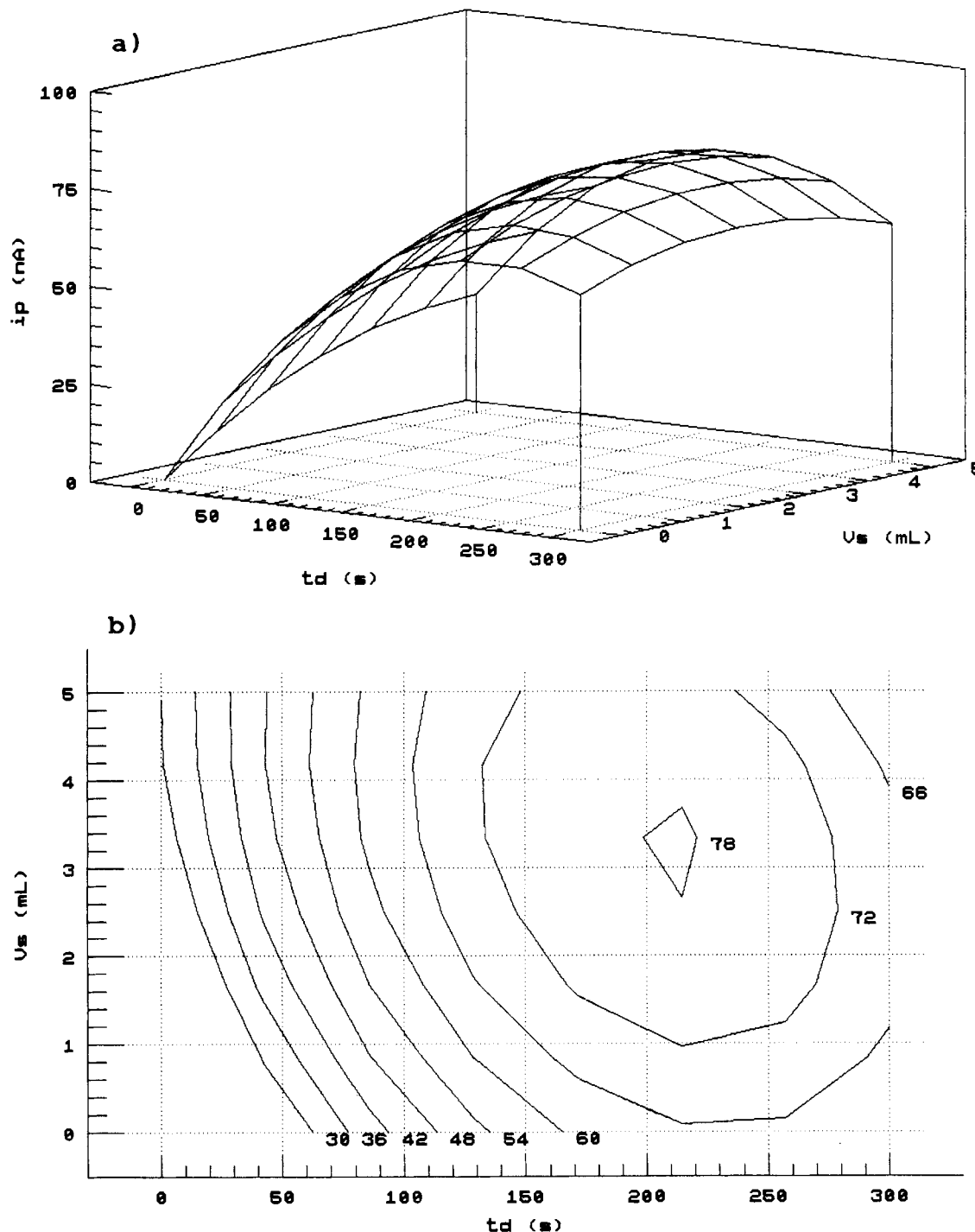
Figure 1. Effect of deposition time and sample volume for digested reserva wine on Zn stripping current (nA): (a) response surface; (b) contour plot.

Instruments. A Metrohm Polarograph equipped with a hanging mercury drop electrode (HMDE), Model 647 VA stand was used, connected to a 646 VA processor. An Ag/AgCl electrode in 3 M KCl was used as reference electrode, the auxiliary electrode being platinum.

Methods. *Treatment of Samples.* (1) *Acid Digestion (Oehme and Lund, 1979).* Forty milliliters of wine, 4 mL of H_2SO_4, and 4 mL of H_2O_2 were brought to boiling point, and every time a darkening of the sample was observed, 4 mL of H_2O_2 was added until the solution remained transparent and clear for at least 5 min. The residue was left to cool and made up to 100 mL with distilled deionized water.

(2) *Acidification to pH 1 (Daniele et al., 1989).* An aliquot of wine was adjusted to pH 1 by HCl addition and left to stabilize for 24 h before measurement.

(3) *Dilution.* An exact volume of wine was diluted to 25 mL with distilled deionized water.

Table I. Characteristics of Wines

wine	aging[a]	pH	extract (g/L)	alcohol content (% v/v)
young		3.20	33.5	12
crianza	12 months in oak barrel	3.15	26.9	12
reserva	12 months in oak barrel and 14 months in bottle	3.21	25.4	12

[a] According to Spanish legislation.

Study of the Influence of V_s and t_d on the Characteristics of the DPASV Signal. For each treatment were taken the following volumes of samples: 0.5, 1, 2, 3.5, and 5 mL. These volumes were diluted to 25 mL with distilled water. Measurements of i_p and E_p were performed directly on these dilutions, except for digested samples to which Na_2CO_3 was added to obtain a pH between 1.8
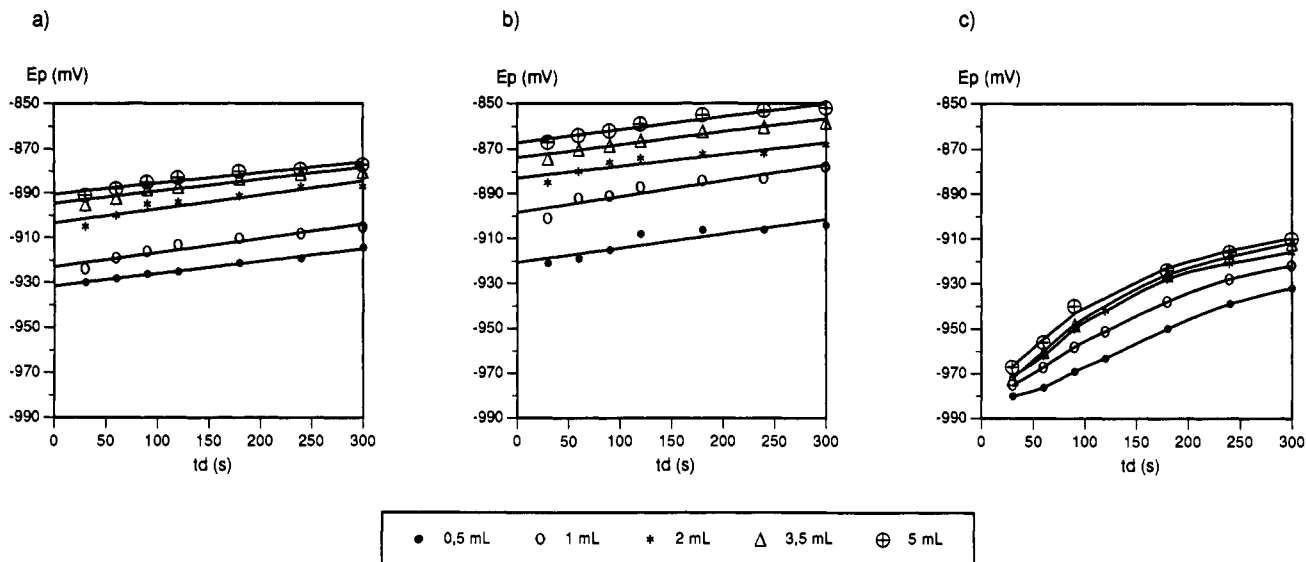
Figure 2. Effect of deposition time and sample volume on Zn stripping potential (mV) of crianza wine: (a) diluted wine; (b) pH 1; (c) acid digestion.
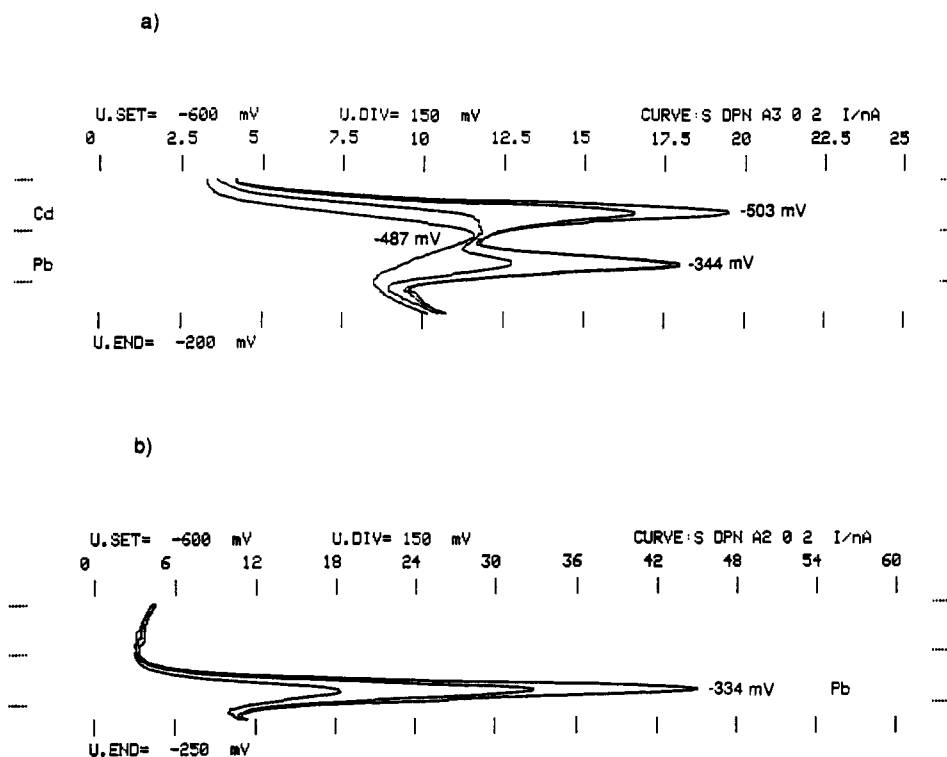
Figure 3. DPASV curves in red reserva wines: (a) Cd and Pb voltammograms of diluted wine before and after two additions of Cd and Pb standards; (b) Pb voltammogram at pH 1 before and after two additions of Pb standards.

and 2.0. For each volume were used deposition times of 30, 60, 90, 120, 180, 240, and 300 s.

Quantitative Determination. Quantitative determination for each wine subjected to all three treatments was performed in the following way:

For total metal content determination (treatment 1), 10 mL of acid-digested sample was diluted to 25 mL with distilled deionized water; solid Na_2CO_3 was added to obtain a pH between 1.8 and 2.0.

Acid-free metal (treatment 2) was measured by acidification of 5 mL of the sample to pH 1 and dilution to 20 mL with distilled deionized water.

Labile metal (treatment 3) was analyzed by taking 5 mL of wine and diluting it to 25 mL with distilled deionized water.

Voltammetric Procedures. The following parameters were used for recording the differential pulse anodic stripping voltammograms: nitrogen deaeration, 5 min; deposition time, (a) for the study of DPASV signal, 30, 60, 90, 120, 180, 240, and

300 s, (b) for quantitative analysis, 30 s for treatments 1 and 2 and 300 s for treatment 3; rest period, 20 s; deposition potential, -1150 mV; mercury drop area, 0.40 mm^2; scan rate, 10 mV/s; pulse amplitude, 50 mV.

Quantifiation was carried out using the standard addition method (two additions per measurement).

RESULTS AND DISCUSSION

Influence of V_s and t_d on DPASV Signal Characteristics. *Zinc.* It was observed in all cases that even with low deposition times and small wine sample volumes, clear, narrow, and well-defined solution peaks appeared. This gives an idea of the lability of this metal and, as will be shown below, the ease of its quantification in any case.

For all treatments, except in the case of digested wine, intensity increased with deposition time and sample

a)   b)   c)
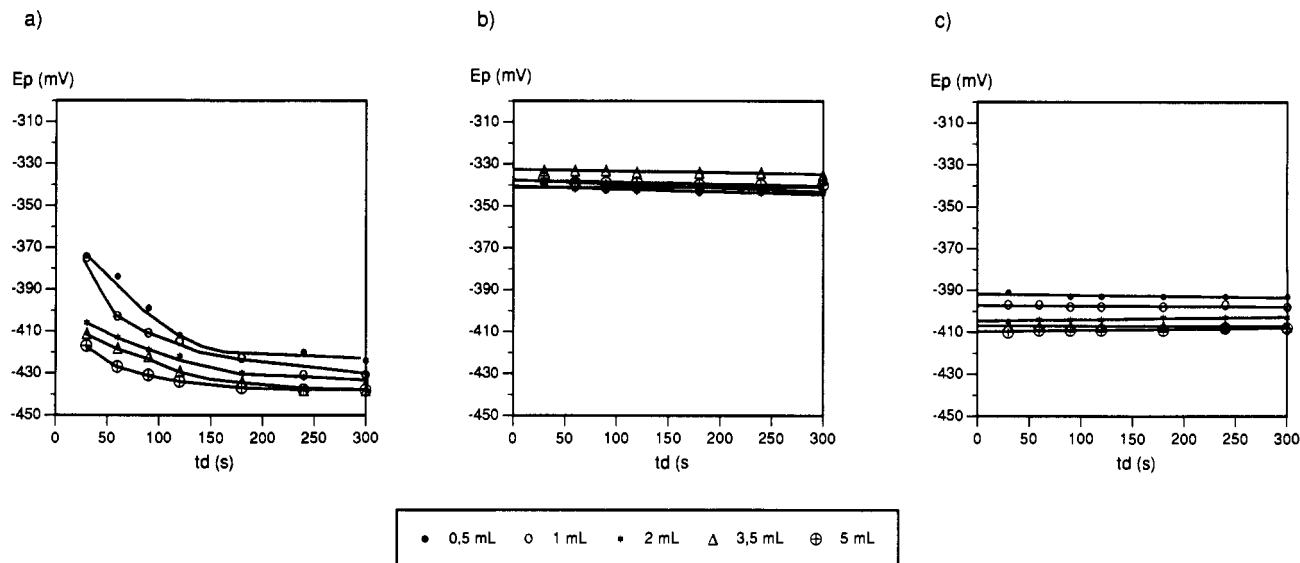
Figure 4. Effect of deposition time and sample volume on Pb stripping potential (mV) of reserva wine: (a) diluted wine; (b) pH 1; (c) acid digestion.

a)   b)   c)
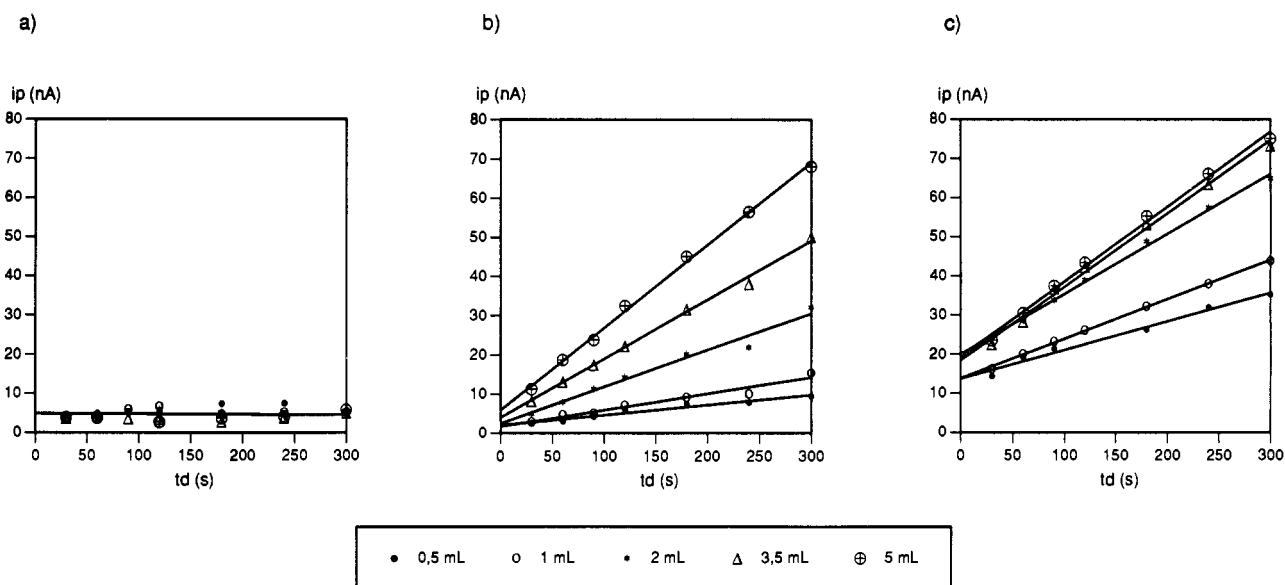
Figure 5. Effect of deposition time and sample volume on Pb stripping current (nA) of crianza wine: (a) diluted wine; (b) pH 1; (c) acid digestion.

volume, as would be expected. The i_p of the digested sample shows variable and unexpected behavior. At relatively small volumes and average deposition times, sharp increases in this parameter followed by decreases were observed. Variation of i_p with deposition time for different volumes of digested reserva wine through the representation of response surfaces (i_p = 0.5652t_d + 12.4294V_s − 0.0012$t_d{}^2$ − 1.2838$V_s{}^2$ − 0.0197$t_d V_s$; r^2 = 0.964) and contours is shown as an example in Figure 1. One explanation of this anomalous behavior is that interferences caused by formation of intermetallic compounds and/or acid matrix may take place. This is because the metals would be free and interaction between these and the electrode would be expected to be very high. To check whether intermetallic compound Cu–Zn (Riley and Watson, 1987; Daniele et al., 1989; Psaroudakis and Efstathiou, 1987; Florence, 1986) was the main reason, the peak height for Zn solutions of different concentrations (4, 8, 16, 24, 40 ppb) was measured at different deposition times (between 50 and 500 s with 50-s intervals). A linear relationship between current and t_d was observed for all concentrations (r^2 ≥ 0.997). Double concentration of Cu

(32 μg/L) was added to the 16 μg/L Zn solution, and it was observed that the same linear relationship remained (r^2 ≥ 0.998). Therefore, it is mainly the digestion acid matrix that provokes this anomalous behavior.

The effect of t_d on E_p is very small for diluted and pH 1 samples. Interference due to adsorption of organic matter on the electrode is irrelevant (in Figure 2a,b the crianza wine is shown as an example). However, for the digested sample (Figure 2c), it was found that E_p began with more negative values than in the previous cases and its variation with t_d toward more positive values was larger, although it always reached values which were less than those obtained in the two cases mentioned above. This would confirm the hypothesis of interaction with the acid matrix.

Cadmium. The cadmium peak does not appear either with a natural pH or with pH 1. In the first case it would be due to the overlapping between lead and cadmium as a wide, unexpected peak appears at very negative potentials, probably from the Pb which would be highly complexed. To confirm this, a known quantity of lead and cadmium was added to a diluted wine sample and it

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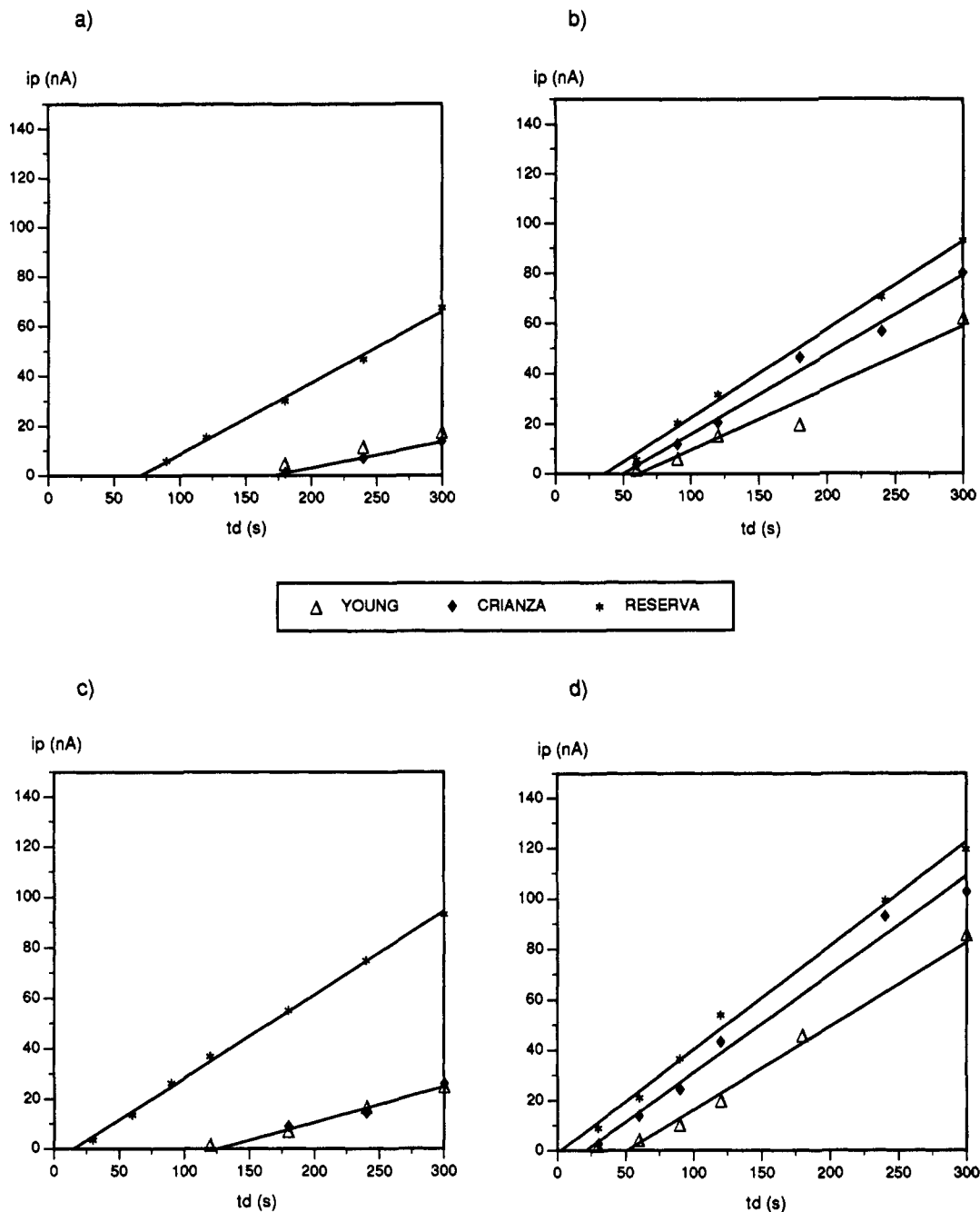
Figure 6. Effect of deposition time on Cu stripping current (nA) for young, crianza, and reserva wines: (a) diluted wine (sample volume 3.5 mL); (b) pH 1 (3.5 mL); (c) diluted wine (5 mL); (d) pH 1 (5 mL).

was observed that both metals produced very close peaks framed within the potential range of the cadmium peak (Figure 3a). However, at pH 1, the same does not appear to happen as the lead produces much narrower and well-defined peaks as well as appearing at slightly more positive potentials (Figure 3b).

In the three digested wines small, although measurable, peaks appear, which would demonstrate the existence of Cd. At pH 1, liberation of this metal is not detected under measurement conditions used. Daniele et al. (1989) managed to determine Cd with a static mercury drop electrode. Spiess et al. (1984) state that at pHs greater than 3 most of the Cd is found complexed by the tartaric and malic acids and, to a lesser extent, by the lactic, citric, and succinic acids present in wine. All this would lead to the conclusion that Cd is found in low concentrations and complexed by compounds present in wine and that, under our measuring conditions, it could only be determined after digestion.

Lead. At natural pH, lead produces very wide peaks. The potential at which they appeared was very negative, overlapped with the Cd peak (Figure 3a), and exhibited variation toward more negative values, both with deposition times and with wine volume (Figure 4a). i_p shows very little variation, as can be appreciated from Figure 5a. These data suggest that lead is found complexed to a great extent, which would make quantitative determination very difficult.

At pH 1, large differences can be appreciated with respect to the previously mentioned behavior. The peaks are narrower (Figure 3b) and of increasing intensity which varies linearly both with time and with sample volume (Figure 5b). This is probably due to the fact that the greater part of the lead at this pH is liberated by the acid (Spiess et al., 1984). The potential, less negative, barely shows variations with time and sample volume (Figure 4b). This would imply that at pH 1 adsorption of organic matter on the electrode does not cause major interference.

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Table II. Concentrations of Zn, Cd, Pb, and Cu, Obtained by DPASV in Three Wines with Different Aging Periods, after Digestion, Acidification at pH 1, and Dilution

wine	treatment	heavy metals (μg/L)			
		Zn	Cd	Pb	Cu
young	digestion	230 ± 6	2.2 ± 0.5	77 ± 6	106 ± 3
	pH 1	230 ± 29	ND[a]	48 ± 3	10 ± 3
	dilution	174 ± 6	ND	b	b
crianza	digestion	342 ± 8	1.3 ± 0.5	92 ± 6	187 ± 15
	pH 1	337 ± 3	ND	69 ± 3	28 ± 3
	dilution	264 ± 22	ND	b	b
reserva	digestion	233 ± 5	1.3 ± 0.4	124 ± 5	185 ± 8
	pH 1	221 ± 9	ND	85 ± 8	40 ± 3
	dilution	190 ± 9	ND	b	59 ± 14

[a] ND, nondetected. [b] Measurements could not be carried out due to the high dispersion of analyses.

In the digested samples, E_p varies very little with respect to t_d and V_s (Figure 4c), while peak current, i_p, increases linearly with deposition time and sample volume (Figure 5c).

It would thus seem that lead is found strongly tied in complexes which dissociate widely at pH 1. The acid matrix produced by digestion does not cause interference in the signal, in contrast to Zn.

Copper. In wines diluted with distilled water, at natural pH, peaks only appeared for the higher sample volumes (3.5 and 5 mL) and high deposition times. This shows the low lability of the cation. Similarly, for the same sample volume, the reserva wine exhibited higher i_p peaks which appear at shorter t_d values. This cation is more labile in the older wine where organic matter is more oxidized (Figure 6a,c). On the other hand, the Cu peaks appear at positive potentials that overlap with the mercury oxidation barrier. Interference could hence be introduced in its determination.

When the sample was acidified to pH 1, peaks appeared for sample volumes over 2 mL. Their intensity increased with deposition time and volume. Their potential shifted markedly toward more positive values with t_d and in lesser proportion with V_s. This would suggest that the organic matter adsorbed would have a considerable influence on the peak of this metal. In these samples, as is the case with the natural pH, older wines produce higher i_p values for the same t_d (Figure 6b,d).

In the digested samples very wide peaks appeared at short t_d and low V_s values which, as t_d increases, result in two peaks which vary their potential inversely. This could be a consequence of the stabilization of Cu(I) with some component of the sample (Cl⁻ ions), which would indicate that the coexistence of both states [Cu(I) and Cu(II)] is possible (Daniele et al., 1989).

Quantitative Determination. Results obtained from the analysis of Zn, Cd, Pb, and Cu for wines studied subjected to all three treatments are shown in Table II in which the total metal content, the metal displaced by the acids, and the labile content are presented. In all wines, Zn is the metal which presents the highest concentrations, followed by Cu, Pb, and, finally, Cd with the lowest levels.

Zn concentration ranged from approximately 230 μg/L, for young and reserva wines, to 342 μg/L for crianza wines. Zn content in diluted samples was between 72 and 81% of the total metal content, and it is an indication of the high lability of Zn, as one would be led to expect from the voltammograms of the samples and the evolution of i_p and E_p (Figure 2a).

Cu is the second element in concentration, with total values between 106 μg/L for the young wine and approximately 186 μg/L for the crianza and reserva wines. Metal

analyzed after acidification varies in concentration depending on the degree of aging of the wine, from 10 to 40 μg/L, which results in a variation with respect to the total of 9.5% for young wine and 21.6% for the reserva. This shows that the influence of the addition of acid on the displacement of balances is greater as the organic matter is more oxidized. It was also observed that measurement of diluted samples is only possible in the case of the reserva wine; in the other two there is either great dispersion of results or the mercury oxidation barrier prevents analysis. Lability is much lower than in Zn. In the reserva wine 32% of the total was found to be labile.

Pb shows levels between 77 and 124 μg/L. The equilibria of this metal are greatly displaced toward free metal after acidification; between 62 and 75% of the total is freed. However, Pb is found strongly complexed and its determination is therefore not possible at the natural pH of wine. Figures 4 and 5 corroborate this behavior.

For Pb and Cu, the results of digested samples provide significantly greater values than those obtained from acidified and diluted samples ($p = 0.05$).

Cd is the metal found in the lowest concentration (1.3–2.2 μg/L). It has only been possible to obtain it after digestion; neither acidification nor dilution has enabled detection of the metal under the measuring conditions used. It can be brought about either by the low concentration of the cation or by the strong retention of Cd by the organic and inorganic ligands present in wine.

The data confirm that, in general, aging increases the lability of studied metals, probably due to the decreasing organic matter content (Table I). Labile Cu is only found in reserva wine (32% of total content), and an increment with aging from 72 to 81% is observed for Zn. A similar effect happens for Cu in the case of acidified samples. For Zn and Pb, the obtained percentage is almost unmodified for the aging of the wine.

ACKNOWLEDGMENT

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