Development of 2-aminobenzoic Acid as a Complexing Ligand for Simultaneous Adsorptive Cathodic Stripping Voltammetric Determination of Trace Copper, Lead and Cadmium

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Abstract
In this study, 2-aminobenzoic acid (ABA) has been used as a complexing ligand for metal ions simultaneous determination by adsorptive cathodic stripping voltammetry (AdCSV). This selective and sensitive method was based on Cu(II), Pb(II) and Cd(II) ions + ABA adsorptive accumulation, by hanging them onto a mercury drop electrode (HMDE), followed by their reduction through differential pulse cathodic stripping voltammetry (DPSCV). Experimental parameters, such as pH, ABA concentration, accumulation time, potential and scan rate, were examined. Under the optimized conditions, linear calibration curves were established for Cu, Pb and Cd concentrations, in the ranges from 5 to 120, 5 to 150 and 5 to 120 ng/mL⁻¹, respectively. Detection limits (LODs) of 2.05, 1.78 and 2.26 ng/mL⁻¹ were obtained for Cu, Pb and Cd, respectively. The proposed method was applied for the determination of these elements in tap and industrial waste water, as real samples.

Keywords: copper; lead; cadmium; 2-aminobenzoic acid; AdCSV; water samples.

Introduction
Pollution by heavy metals, such as Cu, Pb and Cd, is one of the most serious environmental problems [1]. They are highly toxic to both humans and animals, including their nervous, immune, reproductive, and gastrointestinal systems [2, 3]. Sensitive methods for Cu, Pb and Cd trace amounts determination have received much attention, and many techniques have been employed to develop them. Flame atomic absorption spectrometry (FAAS), graphite furnace atomic absorption spectrometry (GFAAS) and inductively coupled plasma-optical emission spectrometry (ICP-OES) are widely used analytical techniques for Pb [4, 5], Cu [4, 6-9] and Cd [4-6, 10-14] detection. Anodic stripping voltammetry (ASV) and AdCSV techniques have shown numerous advantages, including speed of analysis, good selectivity, sensitivity and performance with different matrices [15, 16]. ASV application to samples with complex matrices is limited, due to the interfering components adsorption onto the electrode surface and, consequently, the loss of electrode activity, and irreproducible results. Also, Cu determination by ASV is not
very sensitive, due to the broadness and closeness of the metal anodic peak potential to that of mercury (Hg) oxidation [17]. The main problem with Cu and other heavy metals simultaneous determination by ASV is the formation of intermetallic compounds between this metal and others, especially Cu-Cd and Cu-Zn compounds [18]. Generally, when Cu is present at concentrations higher than those of the ion of interest, the stripping peak of the latter is severely suppressed or is shifted to another potential.

AdCSV is an alternative method to determine trace metals [19, 20]. This technique is based on the adsorptive accumulation of metal complexes with an added ligand onto the electrode, followed by the adsorbed complexes electrochemical reduction [15]. Several chelating reagents, such as oxine [21], 1-(2-pyridylazo)-2,7-dihydroxy naphthalene [22] and thymolphthalene [23], were used for Cd determination. Also, several ligands, such as 2-[2,3,5-triazolylazo]-5-dimethylaminobenzonic [24], salicyalddehyde thiosemicarbazone [25], cyclopentanone thiosemicarbazone [26], morin [27], oxine [28], 5,5-dimethylcyclohexane-1,2,3-trione 1,2-dioxime 3-thiosemicarbazone [29] and α-benzylmonoxime [30], were already applied for Cu and organic compounds determination (eg. morin) [27]. A mixture of dimethylglyoxime and oxine [31], and carbidopa [32], as complexing agents, was reported for Pb voltammetric determination. AdCSV was successfully used for heavy metals individual and simultaneous determination. In previous works, this method for Ni-Cu [33], Pb-Zn [34], Cd-Cu [35, 36], Pb-Cu [23, 27] and Pb-Cu-Cd [37-39] determination, with different ligands, was described. That we know, there is no report about the application of 2-aminobenzoic acid (ABA) as a ligand in metal ions determination using AdCSV technique. Therefore, in this work, an AdCSV procedure for Cd(II), Pb(II) and Cu(II) simultaneous determination, with ABA as a complexing agent, was described. Also, various factors influencing the method were investigated. This method was used for Cd(II), Pb(II) and Cu(II) ions determination in tap and industrial waste water samples.

**Experimental details**

**Apparatus**

A Metrohm 746/747 VA processor, with a three-electrode cell, made of HMDE, Ag/AgCl (saturated KCl), and platinum, as working, reference and counter electrodes, respectively, was used in differential pulse voltammetry (DPV). All reported potentials were referred to the WE. DPV had a pulse amplitude of 50 mV. The pH measurements were performed by a Metrohm 691 pH meter, with a combined glass electrode. A Varian AA-50 atomic absorption spectrometer, equipped with Pb and Zn hollow cathode lamps, was used for AB measurements.

**Chemicals**

All solutions were prepared with doubly distilled water. The stock solutions of 1000 mg/L Cu(II), Pb(II) and Cd(II) were prepared by carefully weighing solid Cu nitrate (NO₃⁻), Pb NO₃ and Cd NO₃ (Merck), and dissolving them in aqueous 0.5 mol L⁻¹ nitric acid (HNO₃). ABA (Merck) solution was prepared by dissolving an appropriate amount of it in 96% ethanol, and diluting it with doubly distilled water, in different 100 mL volumetric flasks.
Procedures
The supporting electrolyte solution (phosphate buffer (PB), with pH 7.4) was transferred into the electrochemical cell and purged with nitrogen (N) for, at least, 4 min. The accumulation potential (0.0 V vs. 3 M Ag/AgCl/KCl) was applied to a fresh Hg drop, while the solution was stirred for a period of 10 s of accumulation time. Then, after equilibration for 10 s, voltammograms were recorded from 0.35 to -0.76 V, with a potential scan rate of 10 mV/s⁻¹ and a pulse amplitude of 50 mV. Afterwards, Cu, Pb and Cd standard solutions aliquots were introduced into the cell, and the obtained solution was deoxygenated with N gas, for 1 min. Then, a DPV was recorded, according to the described procedure, to give the sample peak current. Each scan was repeated five times, with a new drop for each analyzed solution, and the mean value was obtained. Cu, Pb and Cd stripping peaks were registered at about 0.094, -0.319 and -0.520 V, respectively, and their currents were used as the method for measuring the magnitude of their analytical signals. All data were obtained at room temperature. Calibration graphs were prepared taking the peak current against Cu(II), Pb(II) and Cd(II) concentrations.

Results and discussion
Preliminary experiments were carried out using DP-AdCSV to identify the general features that characterized the Cu(II)-ABA, Pb(II)-ABA and Cd(II)-ABA systems behavior on HMDE. Fig. 1a displays DPVs of the PB (pH 7.0) on the HMDE surface, with a scan rate of 10 mV/s⁻¹.

Effect of pH solution
The pH effect on the cathodic stripping peaks current of Cu, Ni, Cd and Pb was studied in the pH range from 6 to 8, in a solution containing 70 ng/mL⁻¹ of Cu(II), Pb(II) and Cd(II), with 0.45 mmol/L⁻¹ ABA, recording DP-AdCS voltammograms onto the HMDE, by adsorptive accumulation, at -0.25 V, for 60 s. Fig. 2 shows the dependence of the peak heights on the pH. As it is seen, the metal ions peak height...
increased in the pH range from 6 to 7.4, and decreased at pH values higher than 7.4 and lower than 6.5. That may be due to the metal ions hydroxocomplex formation and ABA protonation, respectively. In spite of this, the maximum peaks currents were obtained at pH 7.4, for both metal ions that were selected for subsequent experiments.

**Figure 2.** DP-AdCS voltammetric peak current as a function of pH on the HMDE surface, for a solution with 70 ng/mL of Cu(II), Pb(II) and Cd(II) + 0.40 mM ABA, at an accumulation potential of -0.25 V, accumulation time of 60 s and scan rate of 10 mV/s\(^{-1}\).

**Ligand concentration effect**
The dependence of the Cu(II)-ABA, Pb(II)-ABA and Cd(II)-ABA peaks current on the complexing agent concentration was tested. DP-AdCS voltammograms of 70 ng/mL Cu(II), Cd(II) and Pb(II) + increased amounts of ABA (0.2-5.3 mM) were recorded in an aqueous PB with pH 7.4, following the preconcentration of these metal ions + ABA complexes onto the HMDE, at -0.25 V, by adsorptive accumulation, for 60 s. The obtained results (Fig. 3) showed that the Cu(II)-ABA, Pb(II)-ABA and Cd(II)-ABA complexes peaks currents increased along with higher ligand concentrations, up to 0.45 mM.

**Figure 3.** Effect of the ligand concentrations on the determination of 70 ng/mL\(^{-1}\) Cu(II), Pb(II) and Cd(II), with pH of 7.4, accumulation potential of -0.25 V, accumulation time of 60 s and scan rate of 10 Mv/s\(^{-1}\).
Beyond this concentration, the peak height gradually decreased, probably due to some competition between the complexes and the ligand for the adsorption onto the electrode surface. Consequently, an optimum ABA concentration of 0.45 mM was selected for further experiments.

**Accumulation parameters effect**
The charge on the Hg electrode surface plays an important role in the metal complexes absorption efficiency [40]. In its turn, the solution applied potential and composition significantly affects the charge on the Hg electrode. Therefore, in this study, the accumulation potential effect on the stripping peaks current of metal ions + ABA complexes was individually examined, over the potential range from 0.15 to -0.40 V. The stripping peaks current plots of both metal ions, as a function of the preconcentration potential, are shown in Fig. 4A. As it is obvious, the peaks current increased up to about -0.2 for all metal ions, and slightly decreased towards more negative values. This behavior is due to a competition between the complexes and the ligand for the adsorption onto the electrode surface. Subsequently, the accumulation potential of -0.2 V was used in all further DP-AdCSV measurements.

![Figure 4](image)

**Figure 4.** The influence of: A) accumulation potential; B) accumulation time; and C) scan rate on the cathodic adsorption stripping peak current of 70 ng/mL of Cu(II), Pb(II) and Cd(II), in a PB with pH 7.4, + 0.45 mM ABA. Other conditions: A) accumulation time of 60 s and scan rate of 10 Mv/s; B) accumulation potential of -0.2 V and scan rate of 10 Mv/s; and C) accumulation potential of -0.2 V and accumulation time of 65 s.

On the other hand, DP-AdCS voltammograms of 70 ng/mL of Cu(II), Pb (II) and Cd(II), in a PB, + 0.45 mM ABA, were recorded under optimum conditions, following an increased preconcentration time (30-120 s), by adsorptive
accumulation, at -0.2 V. As shown in Fig. 4B, the peak current of all metal ions-ABA complexes increased with the accumulation time. At higher ion concentrations, the adsorptive saturation of the Hg electrode surface (adsorption equilibrium) was reached and, hence, the peak current levelled off. Therefore, an accumulation time of 65 s was selected for further studies. The scan rate effect was also tested in the range from 2 to 30 Mv/s⁻¹ (Fig. 4C). The current (metal ions-ABA) was found constant, at a scan rate higher than 10 Mv/s⁻¹, which was selected for Cu(II), Pb(II) and Cd(II) simultaneous determination.

**Cu(II), Cd(II) and Pb(II) simultaneous determination**

DP-AdCS voltammograms of the metal ions, at various concentrations, were recorded under optimum procedural conditions (Fig. 5).

![HDME DP-AdCS voltammograms with Cu(II), Pb(II) and Cd(II) various concentrations: 0, 5, 20, 50, 60, 70 and 80 ppm, in a PB with pH 7.4, + 0.45 mM ABA, after preconcentration at -0.2 V onto the HMDE, by adsorptive accumulation, for 65 s, at a scan rate of 10 Mv/s⁻¹.](image)

Figure 5. HDME DP-AdCS voltammograms with Cu(II), Pb(II) and Cd(II) various concentrations: 0, 5, 20, 50, 60, 70 and 80 ppm, in a PB with pH 7.4, + 0.45 mM ABA, after preconcentration at -0.2 V onto the HMDE, by adsorptive accumulation, for 65 s, at a scan rate of 10 Mv/s⁻¹.

Rectilinear relations between the peak current (Iₚ) and Cu(II), Pb(II) and Cd(II) concentrations were obtained over the ranges indicated in Table 1. The results confirm the described DP-AdCS voltammetry method reliability for the Cu(II), Pb(II) and Cd(II) simultaneous determination, within the indicated linear ranges.

**Table 1.** Characteristics of the calibration curves of the described DP-AdCS voltammetric method for the Cu(II), Pb(II) and Cd(II) + ABA simultaneous determination, under optimum conditions.

| Metal ions | Linearity range (ng/mL⁻¹) | Linear square equation | R² (corr. coef.) | LOD (ng/mL⁻¹) |
|------------|----------------------------|------------------------|------------------|---------------|
| Cd²⁺       | 5-120                      | y = 1.0412x + 2.5793   | 0.9945           | 2.26          |
| Pb²⁺       | 5-150                      | y = 1.1523x + 4.0683   | 0.9976           | 1.78          |
| Cu²⁺       | 5-120                      | y = 0.958x - 0.36      | 0.9939           | 2.05          |

**Interference studies**

In multi-elemental determinations at the Hg electrode, the effect of the interference between metal ions is potentially important, because they compete for complexation with the same ligand, and for adsorption onto the Hg drop. Moreover, the inter-metallic compounds formation among the investigated metal
ions may cause an error in their determination. Possible interference by other metal ions on the Cu(II), Pb(II) and Cd(II) AdCSV was investigated through the addition of the interfering ion to a solution containing these metals, under optimized conditions. The results of this study are summarized in Table 2. The tolerance limit was defined as the concentration that gave an error of 3% in Cu(II), Pb(II) and Cd(II) determination. Based on the results, it was concluded that the method is almost free from the interference of the foreign ions. However, a 1-fold concentration of Al(III) (aluminum) had a significant influence on Cu(II), Pb(II) and Cd(II) determination, and 2-fold concentrations of Fe(II) (iron) had a major interference in Cu(II) determination. Interference from Al(III) and Fe(II) ions can be minimized by the addition of 400 and 500-fold concentrations of $F^-$ (fluorine) and SCN$^-$ ions (thiocyanate), respectively, if necessary.

**Table 2.** The effect of foreign ions on the determination of 70 ng/mL$^{-1}$ of each metal ion, under optimum conditions.

| Foreign ions | Tolerance limit |
|--------------|----------------|
|              | [W(M)/W(Cu)]  |
|              | [W(M)/W(Pb)]  |
|              | [W(M)/W(Cd)]  |
| NO$_2^-$, Cl$^-$, IO$_3^-$, WO$_4^{2-}$, N$_2$O$_5$, ClO$_3^-$, ClO$_4^-$, Na$^+$, K$^+$, Ca$^2+$, Mg$^{2+}$, HCO$_3^-$, CO$_3^{2-}$, NO$_3^-$, F$^-$, Br$^-$, SCN$^-$, CH$_3$COO$^-$, Cu$^{2+}$, Al$^{3+}$, Fe$^{2+}$ | 1000 | 1000 | 1000 |
| BrO$_3^-$, SO$_4^{2-}$, I$^-$ | 500 | 500 | 500 |
| Ni$^{2+}$, Sr$^{2+}$, Ba$^{2+}$ | 250 | 100 | 100 |
| CN$^-$, Co$^{2+}$, Mn$^{2+}$, Rh$^{3+}$, Ca$^{2+}$, Ce(IV) | 30 | 50 | 50 |
| Zn$^{2+}$ | 2 | 20 | 20 |
| Cd$^{2+}$ | 1000 | 1000 | 1000 |
| Pb$^{2+}$ | 1000 | — | 1000 |
| Cu$^{2+}$ | — | 1000 | 1000 |
| Al$^{3+}$ | 1 | 1 | 1 |

[a] After addition of 400-fold concentrations of $F^-$ ions to the solution; [b] After addition of 500-fold concentrations of SCN$^-$ ions to the solution

**Tap water and waste water samples analysis**

The described DP-AdCS voltammetry method was successfully applied for Cu(II), Pb(II) and Cd(II) simultaneous determination, in a sample of tap water from Semnan, and in industrial waste water samples from alloys producer companies (Table 3).

**Table 3.** Cu(II), Cd(II) and Pb(II) determination in tap and industrial waste water samples, by the DP-AdCS voltammetry method, under optimum conditions, and by FAAS.

| Sample            | Cu(II) (ppm) | Pb(II) (ppm) | Cd(II) (ppm) |
|-------------------|--------------|--------------|--------------|
|                   | AdCSV        | FAAS         | AdCSV        | FAAS         | AdCSV        | FAAS         |
| Tap water [a]     | 0.119        | 0.12         | 0.98         | 1            | 19.8 [b]     | —            |
| Abzar Mahdi Co.   | 0.142        | 0.14         | 1.55         | 1.5          | 0.811        | 0.82         |
| Fenarlol Iran Co. | 0.812        | 0.8          | 1.97         | 2.0          | 0.289        | 0.3          |
| Abkaran Co.       | 9.35         | 9.46         | 1.61         | 1.5          | 0.319        | 0.32         |

[a] Concentrated by 25-fold
[b] Not found, but added 20 ppm and they were found 19.80 ppm, with a recovery percentage of 99.0%
The same water samples were also analyzed for these metal ions determination by FAAS. A comparison of the results obtained by the DP-AdCS voltammetry method with those resulting from FAAS revealed the capability of the stripping voltammetry method for Cu(II), Pb(II) and Cd(II) determination, at trace concentrations. The results correspond to the mean values of five replicate determinations.

Conclusions
A selective and sensitive DP-AdCSV method was developed for Cu(II), Pb(II) and Cd(II) simultaneous determination, as 2-aminobenzoic acid-complexes in water samples, without significant interference from foreign cations and anions, for the first time. The proposed method presented LODs of ng/mL levels for Cu(II), Pb(II) and Cd(II) determination. The method was successfully applied for these metal ions determination in tap water and industrial waste water samples.

Authors’ contributions
E. Zarei: conceived and designed the analysis, contributed with data or analysis tools, performed the analysis and wrote the paper. A. Izadyar: conceived and designed the analysis, collected the data, contributed with data or analysis tools and performed the analysis. A. Asghari: conceived and designed the analysis, contributed with data or analysis tools and performed the analysis. M. Rajabi: other contributions.

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