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A Systematic Procedure to Enhance Reproducibility of SWASV Cycles in the Determination of Toxic Metals in Real Samples

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Abstract

This work presents for the first time a systematic study on the optimization of the electrochemical cleaning time of a mercury film when it is used as a working electrode material in the analysis of toxic metals, such as Pb^{2+}, used as model metal, in real samples by SWASV.

The optimization study for the film's cleaning time aimed at attaining a Pb^{2+} minimum value in the film after the re-oxidation step of the pre-concentrated metal, given the impossibility of complete removal of traces of the electroactive species from the film. This value was kept constant in each concentration range studied ensuring thus that all assays were performed in initial identical conditions.

An assay performed on a synthetic sample was taken as reference. In it, given the absence of matrix effects, and after the electrochemical cleaning step, a direct proportionality was observed between the residual amounts of Pb^{2+} in the film (which for the cleaning time used was never completely removed) and Pb^{2+} concentration in the solution. This fact determined a high correlation between Pb^{2+} peak current and Pb^{2+} concentration which was not observed when real samples (tree leaves) were analyzed. This behavior may result from the presence of the interfering surfactants always present in real samples of complex matrix.

Cleaning time optimization was performed for the following Pb^{2+} concentration ranges in the real samples of complex matrix: 0.006-0.020, 0.020-0.080, 0.060-0.200 and 0.100-0.600 ppb. As expected, in order to obtain identical levels of film's cleaning efficiency, the need for longer cleaning times has been observed for higher concentrations. The optimized cleaning times for the concentration ranges under study were 120, 150, 180 e 300 s, respectively.

Keywords: Mercury film, Cleaning time, SWASV, Real samples, Surfactants, Toxic metals.

1. Introduction

Anodic stripping voltammetry is one of the most sensitive electroanalytical pre-concentration techniques in the detection and determination of traces of metallic elements.[1-3] One of its other main applications is the characterization and determination of different physico-chemical forms in which a given species may present itself (speciation chemical studies), with the possibility of making an exact assessment of its fraction.[4-8]

Other advantages of anodic stripping voltammetry are the use of relatively low cost equipment (when compared to spectroscopic techniques such as atomic absorption spectrometry, for example) in a compact form, enabling transportation, *in situ* use[9-11] and on-line monitoring in flowing systems.[12-14]

Industry[15], environment[16-19] or medicine[20-23] are examples of areas where this technique is being widely used with high recognition for its unique applicability features.

This technique consists essentially on the reduction (pre-concentration or pre-deposition step), of the electroactive species on the surface of a working electrode, by applying a fixed potential, carefully chosen, during a fixed time interval.

After this step, the determination (or quantification) of the studied species follows, also occurring under controlled potential conditions, by means of the application of an anodic potential sweep meant to originate the re-oxidation of the species previously accumulated on the surface of the working electrode. In this way, a direct proportionality between the measured current, associated to the re-oxidation process of each species, and their concentration is obtained. The potential at which the maximum re-oxidation current value occurs (peak current, Ip), is the peak potential, Ep, which is characteristic of each metallic element, thus allowing its identification [24]

If the potential's disturbance is in the form of a square wave[16], then one may refer to this technique as Square Wave Anodic Stripping Voltammetry, or SWASV.[25-29]

In terms of working electrode material used in SWASV, mercury is by far preferred given its high analytical sensitivity, due to a wide cathodic potential interval, an easily renewable surface[24,30], and also to the formation of a reversible amalgam with the metals deposited during the pre-concentration step, that can be easily re-oxidized during the determination step.[31]

Although mercury is an element with recognized and well known toxicity, thus determining, in recent years, some research studies in order to achieve some alternatives for its use, it is a fact that mercury is still one of the most used electrode material for electroanalytical detection and determination of toxic metals as well as other substances.[32-35] In that regard we strongly believe that the results presented in this work may be extremely useful for authors that develop their work in similar areas of investigation.

The mercury film electrode consists of a thin film[36-341] deposited in the form of small drops[31,42], on the surface of an inert conducting base, using a specific solution, such as mercury nitrate, in a concentration that typically ranges from 1 to 5×10^{-5} mol dm^{-3}. A high surface area/mercury volume is thus obtained during pre-concentration which originates a high deposition current and consequently a high concentration of amalgamated metal. Additionally, and during the determination step, the diffusion of metals from the bulk of the thin mercury film to the surface is quite fast, leading to an increase in the analysis' overall sensitivity.

The mercury film may be deposited prior to performing an assay in a solution specifically prepared to this end (*ex-situ* deposition[43,44]), or directly in the assay solution (*in-situ* deposition[45]). In this way, mercury pre deposition step is eliminated, resulting in a decrease of the total analysis time.

Particular attention is due to the support (substrate) used for the deposition of the film: it must show a high electrical conductivity, be chemically inert towards mercury and the analyzed species or used solvent, and be electrochemically inert in the applied potential interval.

Research carried out in this area as shown that one of the best suited materials to meet this purpose is glassy carbon[31,46-48].

When the working electrode in a SWASV assay is a mercury film (deposited in a substrate such as glassy carbon), one must perform its electrochemical cleaning after ending the pre-concentration/determination cycle, in order to remove any metal trace that might not have been re-oxidized during the determination step. This process becomes more necessary as the concentration of the electroactive species increases.

On the other hand, if the sample presents a complex matrix this cleaning process becomes even more essential as a result of the presence of organic molecules (surface active compounds such as

humic acids or proteins - surfactants). These substances are adsorbed at the working electrode's surface[49-52], causing interferences in the diffusional transport of the analyte which difficult or inhibit the accumulation process (formation of the amalgam between mercury and deposited metal) and the re-oxidation of the electroactive species.

A significant decrease in the results reproducibility is thus verified. Also, peak potentials may deviate to more positive values due to an increasing irreversibility of the re-oxidation process. In these cases a sample pre treatment must be considered as an essential procedure in order to reduce the effects of these surfactants. An acid digestion appears to be the most adequate, especially for samples with high content in organic matter.[53]

Environmental samples, biologic fluids or industrial residues, given their high content in dissolved organic matter are examples of media where this problem is of major significance: Film's cleaning time requires therefore a carefully optimization.

In this work, we present the results of a study regarding the optimization of the cleaning time of a mercury film in several Pb^{2+} concentrations ranges, used as a model toxic metal, in real samples of a complex matrix (poplar tree leaves). The procedure is made to ensure the reproducibility of each assay conditions prior to the preconcentration / determination's cycle, ensuring subsequently a greater analysis' accuracy.

2. Experimental

2.1. Chemical and Reagents

The supporting electrolyte in both test and real samples was 0.1 mol dm^{-3} HNO_3 (pH $\simeq$ 1). This solution was prepared from 65% by Merck and ultra pure (Millipore) water with a resistivity greater than 18.2 MΩ.

Test solutions of Pb^{2+} and standard solutions of Pb^{2+} added to real samples in order to obtain the desired concentrations, were prepared from 1000 mg dm^{-3} standard atomic absorption solution of $Pb(NO_3)_2$ from Merck.

To obtain the mercury film, a 2.5×10^{-5} mol dm^{-3} Hg^{2+} solution was prepared from a 1000 mg dm^{-3} standard atomic absorption solution of $Hg(NO_3)_2$, from Merck.

2.2. Instrumentation

Square Wave assays were performed using an AUTOLAB PGSTAT 10 potentiostat. This device was computer driven using the AUTOLAB GPES software (v. 4.6). The working electrode was a mercury film deposited on the surface of a 3 mm diameter vitreous carbon disc. Reference electrode was an Ag/AgCl system saturated with KCl. The auxiliary electrode consisted of a spiral platinum wire sealed with glass. A conventional electrochemical from PAR was used. This cell, adequate for a working volume of about 50 cm^3, was covered with a Teflon lid.

Microwave sample digestion was performed on a microwave low pressure system, by Microdigest 3.6, equipped with three quartz reaction vessels and an internal Vigreux column.

2.3. Procedure

Poplar trees leaves were collected from a chosen location in Lisbon characterized by high traffic intensity following a sampling procedure previously developed.[54]

The samples were previously digested by acid digestion with microwave heating[55-58] in order to minimize the matrix's organic interferences. Before the digestion was performed, leaves were oven-dried at 65 ºC for 96 h, followed by grinding until a fine dust was obtained. A mass of *ca*.2 g of this powder, rigorously weighed to $\pm$ 10^{-5} g, was always used in the digestion procedure as described elsewhere.[55]

Prior to mercury film deposition, the vitreous carbon disc was polished with 0.02 μm grit alumina. Abundant washing with ultra pure water followed this step.

In the synthetic solutions the mercury film was deposited directly from 25 cm^3 of a supporting electrolyte solution with a Hg^{2+} concentration of 2.5×10^{-5} mol dm^{-3}, using -1.0 V for 300 s. The film

was maintained during the assay. After this procedure adequate volumes of 1×10^{-4} mol dm^{-3} Pb^{2+} solution were added to the supporting electrolyte in order to obtain de desired concentration range.

In regard to real samples, after dissolving the residue form de digestion procedure with 20 cm^3 of a 0.5 mol dm^{-3} HNO_3 warm solution, the final volume of the sample solutions (100 cm^3), contained 0.5 cm^3 of the standard atomic absorption solution of $Hg(NO_3)_2$ so that the mercury film could be deposited in situ and maintained trough the assay.

Adequate volumes of Pb^{2+} solutions were added to 25 cm^3 of sample in order to obtained de desired concentrations.

SWASV assays were performed in the presence of dissolved oxygen.[56]

This work's starting point was a sensitivity study of the voltammetric signal obtained in a given Pb^{2+} concentration range. It included the comparison between the electrical signal obtained in synthetic and real samples, using a constant film's cleaning time.

3. Results and Discussion

The voltammetric parameters used in the SWASV assays were previously optimized in order to improve method sensitivity, repeatability and detection limit.[59-61]

Table 1 summarizes the optimized values for all voltammetric parameters, mercury film formation conditions and further experimental conditions.

Table 1 - Optimized experimental conditions for performing SWASV assays

Parameter	Optimized value
Mercury concentration	2.5×10^{-5} mol dm^{-3}
Mercury deposition potential	-1.0 V
Mercury deposition time	300 s
Mercury film cleaning potential	+0.2 V
Mercury film cleaning time after deposition in-situ	120 s
Mercury film cleaning time between each concentration measurement	Parameter under optimization
Deposition potential of the metals under analysis	-1.0 V
Deposition time of the metals under analysis	30 s
Square wave frequency	100 Hz
Square wave amplitude	25 mV
Potential step	2 mV
Suporting electrolyte (Nitric acid) concentration	0.1 mol dm^{-3}
Stirring speed	1000 rpm

Regarding the synthetic sample, the assay performed for Pb^{2+} concentrations of 0.020, 0.040, 0.060 e 0.080 ppb showed that an electrochemical cleaning time of 60 s was not enough to remove all traces of lead on the mercury film. Consequently, the presence of a residual amount of Pb^{2+} in the film, $Ip_{resid.}$, able to produce electric signal, has been observed. The usual method to overcome this situation is to further increase the cleaning time to ensure the full elimination of lead on the film. However, a different approach was followed in this work: the cleaning time was kept constant after performing the pre-concentration/re-oxidation cycles to the remaining concentrations. Keeping this parameter constant, the amount of residual Pb^{2+} on the film was observed to be directly proportional to its initial concentration (r^2 = 0.996). This fact, in conjunction with a constant efficiency upon cleaning the film ensures, thus, the obtention of an excellent correlation between the electrode's signal, Ip, and Pb^{2+} concentration, within the studied concentration range (r^2=1.000), as can be seen in Table 2. Since quantification of the metal in the sample solution was determined by means of a calibration method, based on the correlation between Ip and Pb^{2+} concentration, this approach is, thus, adequate (for the synthetic sample).

Table 2 - Results obtained on synthetic and real sample assays. Studied concentration range: 0.020 - 0.080 ppb

	Pb^{2+} (ppb)	Ip (μA)		Ip_{resid} (μA)		Film's cleaning efficiency (%)	
		Synthetic	**Real**	**Synthetic**	**Real**	**Synthetic**	**Real**
	0.020	0.76	9.41	0.011	0.632	98.5	93.3
	0.040	1.36	10.04	0.024	0.630	98.2	93.7
	0.060	1.99	11.55	0.042	1.066	97.9	90.8
	0.080	2.62	12.75	0.055	0.856	97.9	93.3
Correlation (r^2)		1.000	0.978	0.996	0.469		
Average	0.050					98.1	92.8
SD						0.29	1.3

Table 2 also shows the results obtained for a similar assay under the same conditions and using a real sample. As for the synthetic sample, as referred, it can be seen that a cleaning time of 60s between two concentration assays was not enough to remove all traces of Pb^{2+} resulting from pre-concentration/re-oxidation cycle. In the case of real sample, the correlation between Pb^{2+} Ip_{resid} and its original concentration is very poor ($r^2 = 0.469$). This is probably due to effects related with the complex nature of the real samples' matrix, *e.g.* adsorption of organic molecules at the surface of the working electrode which hinders, in a randomly manner, the processes of the amalgam formation between mercury and the deposited metal, the re-oxidation of the electroactive species and also the electrochemical removal of the metal during the cleaning process of the mercury film.

This observation, in conjunction with the irreproducibility of efficiency of the mercury film's cleaning efficiency along the concentration range, significantly lowers linearity between Pb^{2+} peak current and concentration ($r^2 = 0.978$) when comparing with synthetic samples. The observed average efficiency for the cleaning process is also lower than that obtained for the synthetic sample probably due to matrix effects.

In practical terms the main consequence of this observation is the impossibility of achieving a thorough quantification of the amount of metallic element present in the samples through linear

regression methods such as calibration curves or standard addition which are commonly used in this type of electroanalytic assays.[62, 63]

The results obtained lead us to conclude that mercury film cleaning time is indeed an important parameter if one means to improve sensitivity, repeatability and detection limit in the SWASV determination of toxic metals in the studied environmental samples. As such, we optimized it using several Pb^{2+} concentration ranges thus ensuring an almost identical starting condition for all assays. The concentration ranges used were: 0.006-0.020 ppb (# 1), 0.020-0.080 ppb (# 2), 0.060-0.200 ppb (# 3) e 0.100-0.600 ppb (# 4). These values resulted from the direct addition, to a real sample volume of 25 cm^3, of adequate volumes of a Pb^{2+} standard solution of 1×10^{-5} mol dm^{-3} (in order to obtain the concentration in #1) e 1×10^{-4} mol dm^{-3} (for all other concentration ranges).

Mercury cleaning time was first optimized for all concentration ranges using the highest concentration in each interval in order to ensure that the value chosen for this particular parameter would lead, in each case, to an identical mercury film cleaning condition to the lower concentrations. This procedure allowed that each pre-concentration/determination cycle could be initiated with the highest possible similarity.

The results are shown in Fig. 1, where for the higher concentration in each range, and after the respective pre-concentration/determination cycle, the variation of $Ip_{resid.}$ for Pb^{2+} against the mercury film cleaning time is plotted.

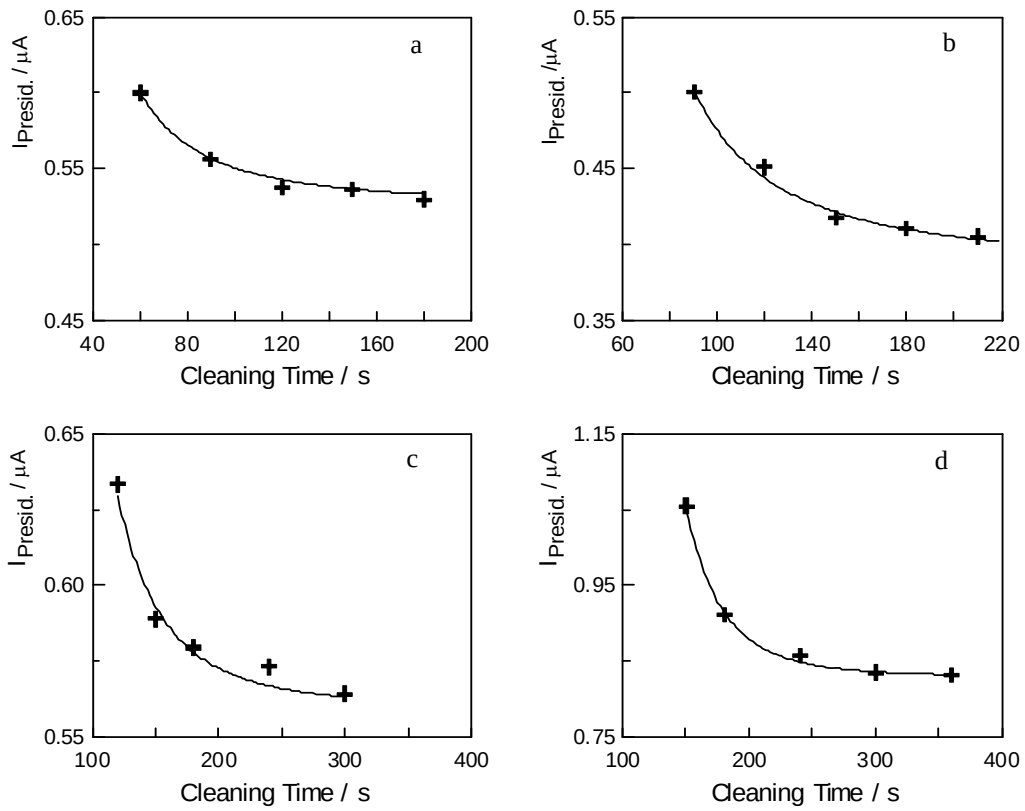

Fig. 1 - $Ip_{resid.}$ for Pb^{2+} *vs.* Mercury film cleaning time. Concentrations under study a: 0.020 ppb, b: 0.080 ppb, c: 0.200 ppb and d: 0.600 ppb.

From the plots in Fig. 1 we can observe that in all cases some residual Pb^{2+} was present since an electric signal was always obtained after successive cleaning. Therefore, it was decided to use, for each concentration, a constant cleaning time, chosen in such way that no significant decrease in the amount of residual Pb^{2+} present could be observed. This criterion is also linked to the goal of not increasing considerably global analysis time. As expected, longer cleaning times were needed for the higher concentrations.

Mercury cleaning times selected through these assays were tested afterwards by studying the electrode response time in real samples along several Pb^{2+} concentration ranges. Thus, in the 0.006 to 0.020 ppb interval, to which corresponds an average concentration of 0.010 ppb a cleaning time of 120 s was used. In the 0.020 to 0.080 ppb interval (average concentration of 0.050 ppb) a cleaning time of 150 s was used. In the 0.060 to 0.200 ppb range (average concentration of 0.110 ppb) a

cleaning time of 180 s was used. The time selected for the 0.100 to 0.600 ppb range was 300s, to which corresponds an average concentration of 0.300 ppm.

These assays lead to the expected results. It was observed that for each concentration range the selected time was sufficient to obtain a single (minimum) residual amount of Pb^{2+} in the film for all concentrations of that given range, ensuring the desired reproducibility for the preconcentration/reoxidation cycles. Indeed, in all cases a high correlation was observed between Ip and Pb^{2+} concentration. Table 3 summarizes these results for all concentration ranges.

Table 3
Results of the tests on selected time for mercury film cleaning in real samples. Sandard deviation values for cleaning efficiency average are indicated between ().

	Pb^{2+} concentration range (ppb)	Film cleaning time (s)	Ip (µA)	Ip_{resid} (µA)	Cleaning efficiency (%)
#1	0.006		6.70	0.660	90.1
	0.008	120	6.87	0.658	90.4
	0.010		7.02	0.645	90.8
	0.020		7.70	0.653	91.5
	Average = 0.010		r^2 = 0.999		Average = 90.7 (0.61)
#2	0.020		17.49	2.279	87.0
	0.040	150	18.20	2.185	88.0
	0.060		19.20	2.185	88.6
	0.080		20.03	2.153	89.3
	Average = 0.050		r^2 = 0.995		Average = 88.2 (0.97)
#3	0.060		6.31	0.669	89.4
	0.080	180	6.58	0.666	89.9
	0.100		7.12	0.668	90.7
	0.200		8.75	0.679	92.2
	Average = 0.110		r^2 = 0.995		Average = 90.1 (1.22)
#4	0.100		5.56	0.866	84.4
	0.200	300	7.23	0.859	88.1
	0.400		9.65	0.858	91.1
	0.600		13.00	0.858	93.4
	Average = 0.300		r^2 = 0.995		Average = 89.3 (3.90)

On Figures 2a and 2b we can see, as an example, the plot of the calibration lines and voltammograms for Ip and (respective Ip_{resid}) of Pb^{2+} obtained from the assays performed on the first (# 1) and fourth (# 4) concentration ranges.

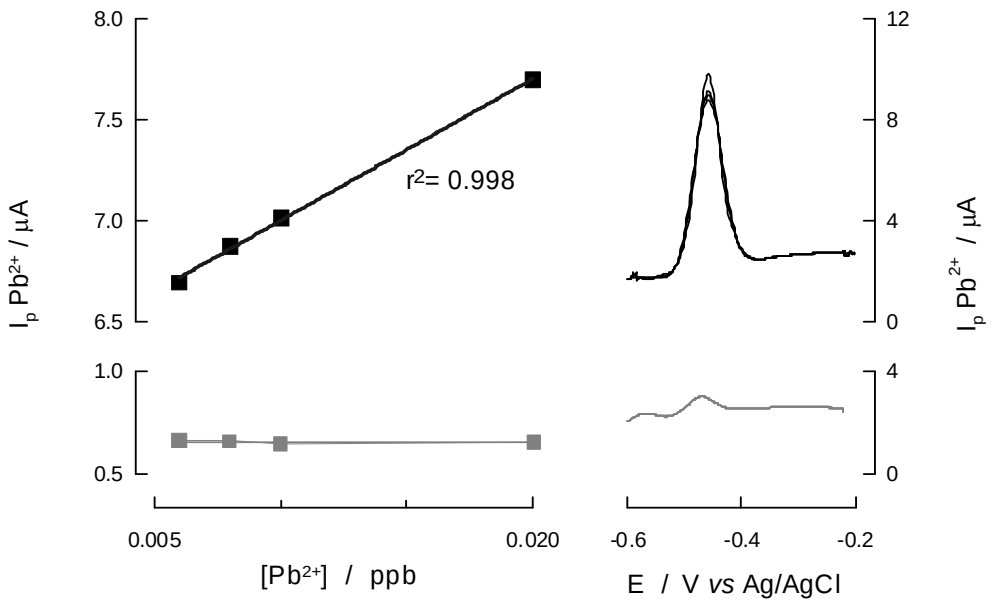

Fig. 2a - Calibration lines and voltammograms obtained for concentration range # 1.

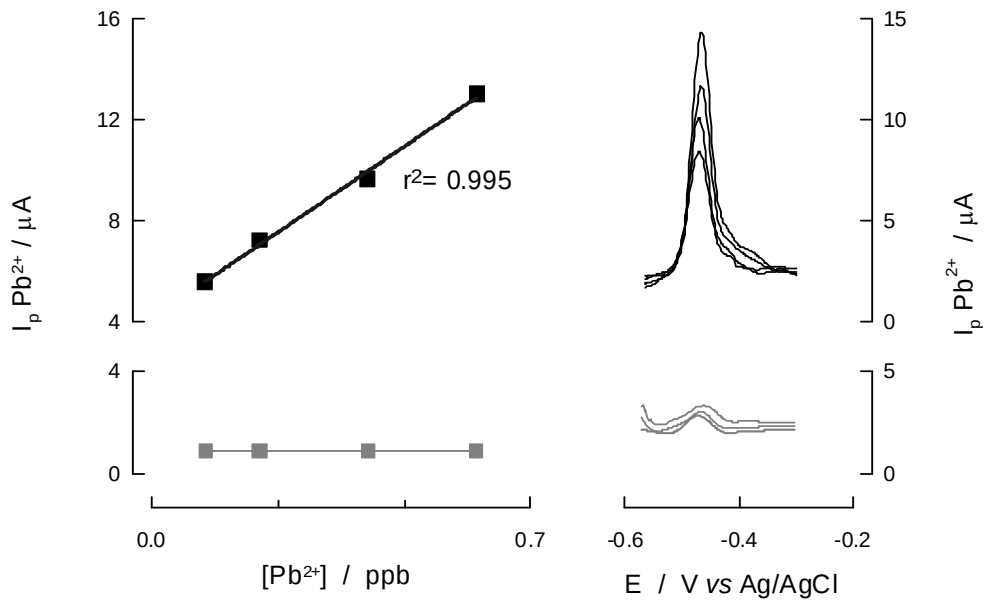

Fig. 2b - Calibration lines and voltammograms obtained for concentration range # 4.

Fig. 3 shows the overall relation between average Pb^{2+} concentration, mercury film cleaning time and mercury film cleaning efficiency.

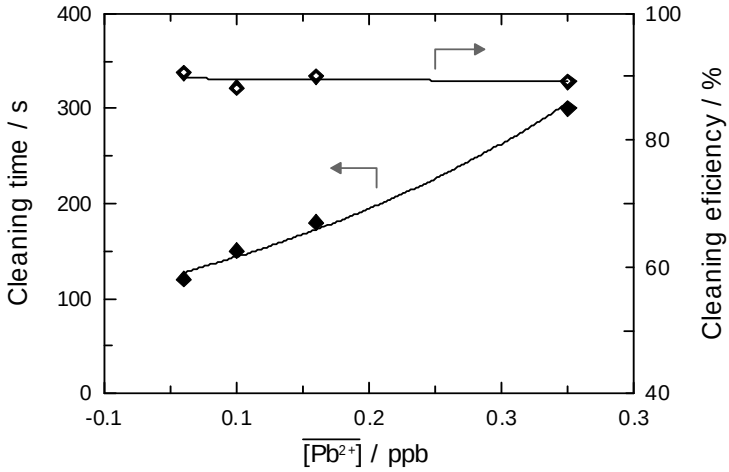

Fig. 3 - Crossing data bettween average Pb^{2+} concentration, mercury film cleaning time and mercury film cleaning eficiency.

4. Conclusions

The results obtained clearly show that the cleaning time of a mercury film is a highly significant experimental variable in trace metal analysis by SWASV, whose value needs to be carefully optimized mostly when dealing with real samples of a complex matrix.

This need becomes even more important when quantification assays are performed, by means of linear regression methods, and the same mercury film is used throughout the assay. The main advantage of this late aspect concerns the reduction of analysis time associated with the need of mercury film deposition prior to ach cycle.

As shown by our results, a strategy aiming to increase the analysis accuracy may go through an optimization of mercury film's cleaning time, established on the basis of the highest concentration value of the working range(s). Only in this way can one ensure that the assays regarding each other concentration value will take place under the same experimental conditions, ensuring, thus, a greater analysis' reproducibility and accuracy.

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