Talanta 93 (2012) 301–306

Contents lists available at SciVerse ScienceDirect

Talanta

journal homepage: www.elsevier.com/locate/talanta

Development of acid phosphatase based amperometric biosensors for the inhibitive determination of As(V)

S. Sanllorente-Méndez*, O. Domínguez-Renedo, M.J. Arcos-Martínez

Department of Chemistry, Faculty of Sciences, University of Burgos, Plaza Misael Bañuelos s/n, 09001 Burgos, Spain

ARTICLE INFO

Article history:
Received 23 November 2011
Received in revised form 7 February 2012
Accepted 16 February 2012
Available online 22 February 2012

Keywords:
Acid phosphatase
Enzymatic biosensor
As(V) determination
Screen-printed electrodes

ABSTRACT

An enzymatic amperometric procedure for the direct measurement of As(V) in the presence of As(III) was developed. The method is based on the inhibitive action of this species on acid phosphatase enzyme (AcP) activity. Screen-printed carbon electrodes (SPCEs) were used as support for the cross-linking immobilization of the enzyme AcP. 2-Phospho-L-ascorbic acid was used as a novel substrate, in arsenic determination, which amperometric response decreased by the presence of As(V) ions. The optimum working conditions were found using experimental design methodology. Under these conditions, repeatability and reproducibility of the constructed biosensors were determined, reaching values below 8% in terms of residual standard deviation. The capability of detection obtained for As(V) was 0.11 μM for AcP/SPCE biosensors. Analysis of the possible effect of the presence of foreign ions in the solution was performed. The method was successfully applied to the determination of the As(V) content in a ground water sample.

1. Introduction

Arsenic is an ubiquitous toxic element present in trace concentration in the environment; its mobilization or redistribution is influenced by both abiotic and biotic processes. The contamination of surface or ground water by arsenic represents a threat to human health, taking into account that drinking water normally derives from both types of sources. In fact, there are several parts of the world where high levels of arsenic are present in water supplies [1]. Symptoms of chronic exposure to arsenic in drinking water are numerous including several types of cancer, particularly of skin and lung, as well as cardiovascular, renal, hematological and respiratory disorders [2–5]. Therefore, arsenic pollution may be regarded as a global issue.

Arsenic occurs in the environment in several chemical forms, showing different toxicological characteristics. Organic forms of arsenic are rarely significant in ground water, however, the inorganic forms: arsenite (AsO_3^{3-}) and arsenate (AsO_4^{3-}) are often found in this kind of water. The concentration of As(V) in natural waters is much higher than that of As(III) [6]. Therefore, the development of an analytical method for the specific determination of this arsenic species is of great interest.

Many techniques have been described in the literature for the determination of arsenic in ground water. These included inductively coupled plasma atomic emission spectrometry (ICP-AES) [7], inductively coupled plasma mass spectrometry (ICP-MS) [8–12], graphite furnace atomic absorption spectrometry (GFAAS) [13], hydride generation atomic absorption spectrometry (HGAAS) [14,15], and hydride generation atomic fluorescence spectrometry (HG-AFS) [16]. These methodologies are often laboratory-based and time-consuming and may lead to large capital cost for multisample analysis. But, its major drawback is that these methods are unable to distinguish between As(III) and As(V) in the analyzed samples.

There are few proposed methods to the direct determination of As(V), without pretreatment of samples. Recently, a colorimetric method using the molybdenum blue complex has been developed for the sensitive determination of As(V) [17].

Electrochemical biosensors have been increasingly developed for in situ monitoring in environmental and health care applications, due to their advantages in terms of simplicity, portability, short response time, sensitivity, and high selectivity due to substrate specificity [18]. In this way, Cosnier et al. [19] have developed a bienzymatic biosensor for the specific determination of As(V) based on the inhibitory effect of this species on the acid phosphatase enzyme (AcP). This amperometric biosensor was constructed by the simultaneous entrapment of AcP and polyphenol oxidase (PPO) enzymes into anionic clays (layered double hydroxides) on a glassy carbon electrode surface. In spite of the high sensitivity of this method, the development of the described biosensor results very tedious and time-consuming, being necessary the immobilization of two different enzymes.

* Corresponding author.
E-mail addresses: silsan@ubu.es (S. Sanllorente-Méndez), olgado@ubu.es (O. Domínguez-Renedo), jarcos@ubu.es (M.J. Arcos-Martínez).

0039-9140/$ – see front matter © 2012 Elsevier B.V. All rights reserved.
doi:10.1016/j.talanta.2012.02.037

In this work a simply amperometric biosensor based on the immobilization of the enzyme AcP on a screen-printed carbon electrode (SPCE) is reported. The use of screen-printed electrodes (SPEs) increases the performance of the developed biosensor due to their disposable features and high reproducibility, together with their low cost of production and their great versatility [20–23]. To the best of the authors' knowledge, this is the first time that a disposable AcP biosensor (AcP/SPCE) has been used for the high selective and sensitive determination of As(V), being also the first time that 2-phospho-L-ascorbic acid has been used as an AcP substrate in As(V) analysis.

2. Experimental

2.1. Reagents

Several inks were used in the production of screen-printed electrodes, namely Electrodag PF-407 A (carbon ink), Electrodag 6037 SS (silver/silver chloride ink) supplied by Achenson Colloiden (Scheemda, The Netherlands). D2071120D1 (dielectric ink) provided by Gwent Electronic Materials (Torfaeen, UK).

All solutions were prepared with water purified with a Milli-Q device which provided a conductivity of 0.05 μS/cm.

AcP (E.C.3.1.3.2, type IV-S from potato, 4.6 U/mg, Sigma, Steinheim, Germany), glutaraldehyde (GA) (Sigma, Steinheim, Germany), bovine serum albumine (BSA) (Sigma, Steinheim, Germany) and 2-phospho-L-ascorbic acid (Sigma, Steinheim, Germany) were used.

Arsenic acid (H_3AsO_4, 1002 ± 5 mg L^{-1}) solution CertiPur® was obtained from Merck (Darmstadt, Germany).

100 mM acetate buffer (Panreac, Barcelona, Spain) and 10 nM $MgCl_2$ (Merck, Darmstadt, Germany) solution were used as supporting electrolyte. NaOH (J.T. Baker, Deventer, The Netherlands) was used to adjust the pH value.

2.2. Apparatus and software

Cyclic voltammetric and chronoamperometric measurements were performed with an AUTOLAB PGSTAT 12 potentiostat with GPES software (Eco Chemie, Utrecht, The Netherlands). Screen-printed three-electrode strips were produced using the DEK 248 screen-printing system (Weymouth, UK), using carbon ink for the working and auxiliary electrodes and Ag/AgCl ink for the reference electrode.A rotary evaporator (ILMVAC, Ilmenau, Germany) was used for sample preconcentration.

The pH of the solutions was measured with a Crison Model 2002 (Barcelona, Spain) pH meter.

Data analysis was processed with a NEMRODW [24] software package for the experimental design process, and PROGRESS [25] for the robust regression.

3. Methods

3.1. SPEs preparation

Home-made SPCEs were used in the determination of As(V). For the construction of the SPCEs successive layers of different inks were printed onto a polyester strip substrate following the printing procedure described in previous works [26–28].

Before using the SPCEs, working and counter electrodes were polished with a SiC-paper No. 4000 disc (Struers, Copenhagen, Denmark). Then, the working electrode surface (4 mm^2) was activated by recording 20 cycle voltammograms between 2 V and −2 V, scan rate, 100 mV s^{-1}, in a 0.1 M KCl solution.

3.2. AcP immobilization in SPCEs

AcP was immobilized by cross-linking with BSA and GA. The optimum immobilization process was reached by mixing 5 μL of a 6.64% (w/v) BSA and 5 μL of a 0.6% (w/v) AcP solution with 10 μL of a 2.5% (w/v) GA solution. Then, 5 μL of this mixture was dropped onto the working electrode surface. The electrode was kept at 4 °C for 1 h. The excess of GA in the insoluble biocomponent was thoroughly eliminated by rinsing with acetate buffer. Finally, it was left to get dried at room temperature.

3.3. As(V) determination procedure

The AcP/SPCE biosensor was placed in the electrochemical cell containing 5 mL of buffer solution. An adequate potential was applied and, once a steady-state current was set, a defined amount of substrate solution was added to the measuring cell. A large oxidation current was observed due to the addition of substrate, and a plateau corresponding to the steady-state response was reached. Then, fixed portions of the As(V) stock solution were added consecutively reaching, each time a plateau. Each addition of As(V) solution resulted in a current decrease proportional to the amount of As(V) added.

Enzyme electrodes were conditioned in a stirred buffer solution for 5 min between each calibration setting.

4. Results and discussion

In order to develop an AcP/SPCE biosensor for the determination of As(V), two different AcP substrates, namely, phenyl phosphate and 2-phospho-L-ascorbic acid, were studied.

The amperometric response obtained for phenyl phosphate presents a lack of stability as it can be seen in Fig. 1a. This amperometric signal observed is related to the oxidation of the product of the enzymatic reaction (phenol). The decrease in the steady-state current may be ascribed to fouling of the electrode by electroinactive polymers that resulted from the polymerization of electrochemically oxidized phenol. Consequently, this biosensor was totally unsuitable for the amperometric determination of As(V) using phenyl phosphate as a substrate.

2-Phospho-L-ascorbic acid was then tested as a suitable AcP substrate for the determination of As(V) using an AcP/SPCE biosensor. This substrate is not toxic and, moreover, it is commercially available at a low cost [29–31]. The amperometric response obtained by AcP/SPCE electrodes when 2-phospho-L-ascorbic acid was used as substrate resulted highly stable (Fig. 1b). For this reason it was selected for next experiments.

Cyclic voltammetry has been used in order to study the behavior of the selected substrate on SPCEs.

In a first step, the cyclic voltammetric response of 2-phospho-L-ascorbic acid was analyzed using both electrodes, SPCEs and AcP/SPCEs. Fig. 2 shows that the substrate has a different behavior in the presence or absence of the enzyme at the electrode.

The voltammetric peak obtained at 0.67 V, when bare SPCEs were used (Fig. 2a), was due to the oxidation of 2-phospho-L-ascorbic acid. This peak was not observed when AcP/SPCEs were utilized (Fig. 2b). In this last case, the AcP enzyme promotes the hydrolysis of 2-phospho-L-ascorbic acid to L-ascorbic acid, which can be oxidized on the SPCE surface at a potential of 0.01 V.

Next, the influence of As(V) in the 2-phospho-L-ascorbic acid voltammetric signal obtained in an AcP/SPCE was analyzed. As it can be seen in Fig. 3a, two oxidation peaks are observed for 2-phospho-L-ascorbic acid. The first one, observed at 0.01 V, can be attributed to the oxidation of L-ascorbic acid as it has been described in Scheme 1. The second oxidation peak (0.67 V) is

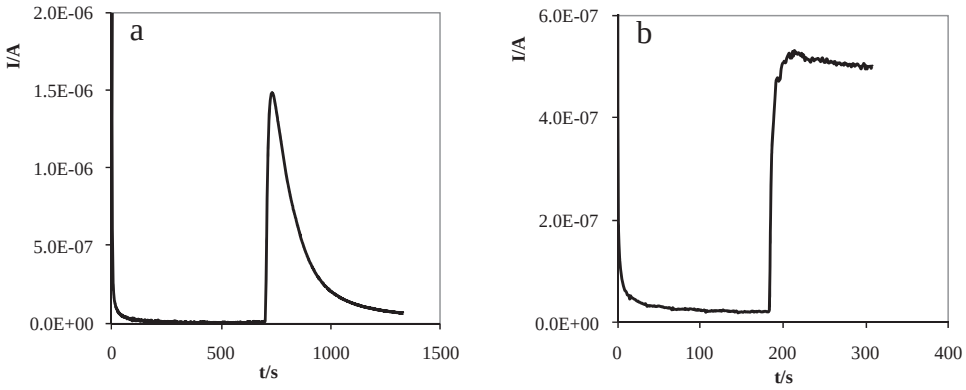


Fig. 1. Amperometric signal obtained at a AcP/SPCE. (a) Phenyl phosphate E_{app} +0.9 V, (b) 2-phospho-L-ascorbic acid E_{app} +0.4 V. Acetate buffer pH 6 and 10 mM $MgCl_2$.

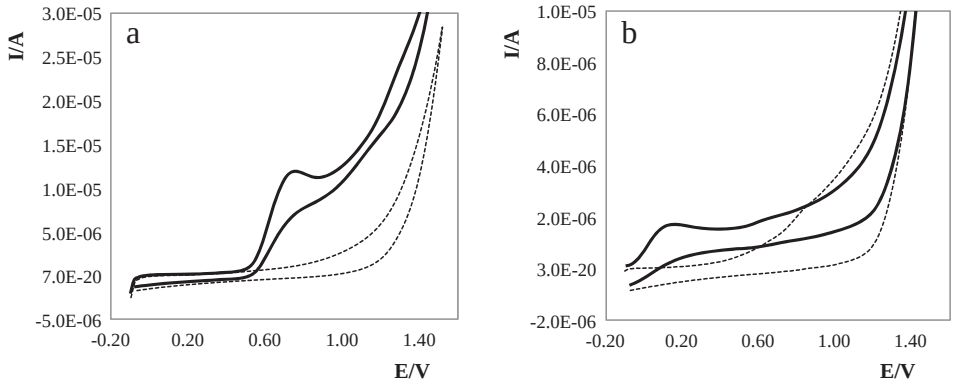


Fig. 2. Cyclic voltammograms for 4 mM 2-phospho-L-ascorbic acid solution in acetate buffer pH 6.0 and 10 mM $MgCl_2$. Scan rate 100 mV/s. (a) SPCE and (b) AcP/SPCE.

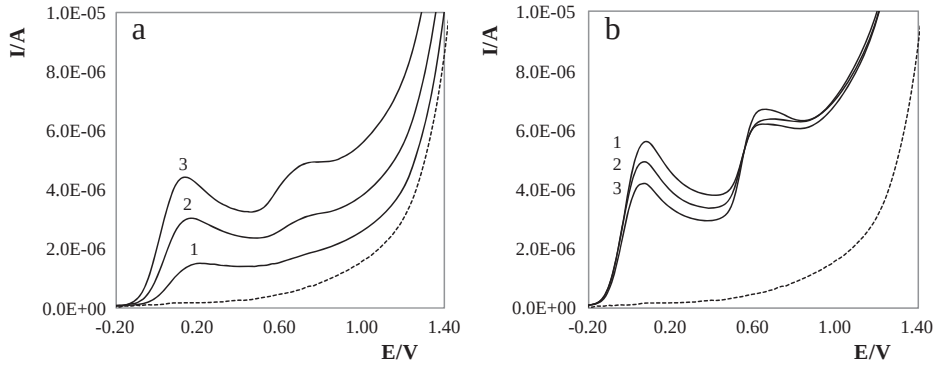
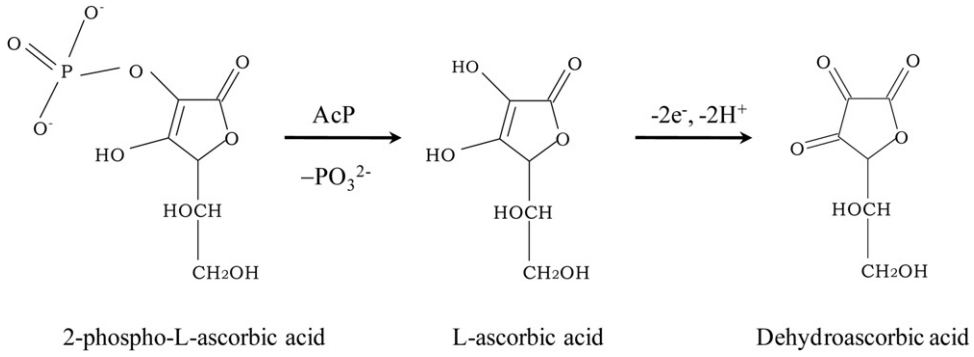


Fig. 3. Cyclic voltammograms obtained at a AcP/SPCE in acetate buffer pH 6.0 and 10 mM $MgCl_2$. Scan rate 100 mV/s. (a) 2-Phospho-L-ascorbic acid additions (1) 4 mM, (2) 8 mM and (3) 16 mM and (b) As(V) additions (1) 0, (2) 0.2 mM and (3) 0.4 mM.



Scheme 1. Mechanism of the enzymatic hydrolysis of AcP enzyme.

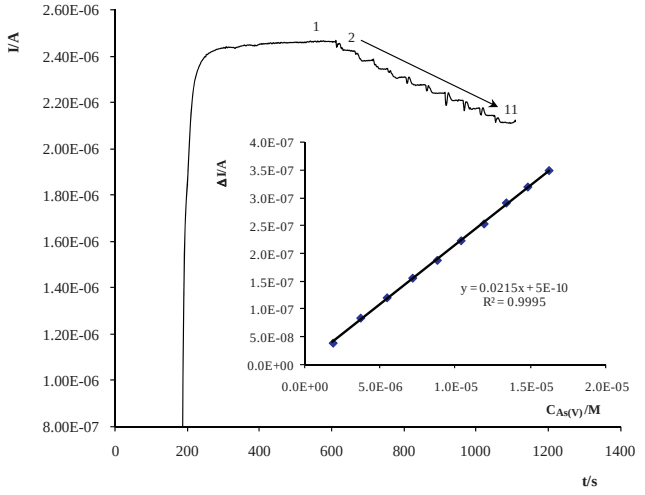


Fig. 4. Cronoamperometric curve recorded for a substrate concentration (1) 3.68 mM at a AcP/SPCE and a consecutive injecting of portions of As(V) into the cell to give an overall concentration of: (2) 1.9 μM, (3) 3.7 μM, (4) 5.4 μM, (5) 7.2 μM, (6) 8.8 μM, (7) 10.4 μM, (8) 11.9 μM, (9) 13.4 μM, (10) 14.8 μM and (11) 16.2 μM. E_{app} +0.33 V. Acetate buffer pH 5.25 and 10 mM $MgCl_2$. The inset shows the relative calibration plot.

observed at high concentrations of substrate and it can be related to the direct oxidation of this compound on the electrode. Successive additions of As(V) cause a reduce in the first oxidation peak, due to the inhibitory effect of this species on the enzymatic activity of AcP. Therefore, the use of 2-phospho-L-ascorbic acid is presented as a new and very interesting alternative in the development of amperometric biosensors to the determination of As(V).

The AcP/SPCE amperometric biosensor, constructed following the above-described procedure, was very sensitive to the concentration of As(V), as it can be seen in Fig. 4. In this figure, it is shown that, as result of the inhibition of AcP enzyme by As(V), the concentration of the generated L-ascorbic acid decreased, which provoked the diminution of the analytical signal obtained for 2-phospho-L-ascorbic acid.

As(V) inhibition action was quantitatively evaluated determining the difference between the steady-state current in the absence of As(V) (I_0) and the steady-state current in the presence of As(V) (I). The parameter $\Delta I(I_0 - I)$ depends on 2-phospho-L-ascorbic acid concentration, applied potential (E_{app}) and pH of the buffer solution. Thus, it is necessary to optimize all of these variables in order to ensure the quality of the results.

Experimental designs have been used as a tool for optimization. In this case, 2^k (k = number of variables) central composite designs were applied, with replication in the central point in order to estimate the experimental error. The response to be optimized was the ΔI obtained for a sample containing a concentration of As(V) of 10 μM.

The first phase in the optimization process involved a 2^3 central composite design. The values of the experimental variables were selected taking into account the optimum pH of enzyme and the potential peaks of substrate and their hydrolysis product. This first experimental design allowed selecting a narrower range of experiments to obtain optimal values of the influent variables that give the maximum response. Then, a second 2^3 central composite design was carried out. The high (+) and low (−) and the central point (0) for each factor were as follows:

E_{app} (+) = 0.3 V	pH (+) = 5.5	$C_{substrate}$ (+) = 3.6 mM
E_{app} (−) = 0.1 V	pH (−) = 5.0	$C_{substrate}$ (−) = 2.0 mM
E_{app} (0) = 0.2 V	pH (0) = 5.25	$C_{substrate}$ (0) = 2.8 mM

From analysis of the variance (ANOVA) in Table 1, it may be deduced that E_{app} (B) and $C_{substrate}$ (C) can significantly influence the response. Furthermore, pH (A) is not a significant factor and can, therefore, be fixed.

Table 1
ANOVA with the data of the 2^2 central composite design for the optimization of experimental variables in As(V) determination.

Effect	SS[a]	DF[a]	MS[a]	F_{ratio}[a]	P_{level}[a]
A: pH	530.15	1	530.15	4.37	0.1717
B: E_{app}	13473.00	1	13473.00	111.04	0.0089*
C: $C_{substrate}$	2472.90	1	2472.90	20.38	0.0457*
AA	441.53	1	441.53	3.64	0.1967
AB	162.00	1	162.00	1.34	0.3673
AC	220.50	1	220.50	1.82	0.3100
BB	14.44	1	14.44	0.12	0.7630
BC	112.50	1	112.50	0.93	0.4372
CC	825.34	1	825.34	6.80	0.1209
Lack of fit	3085.73	5	617.15	5.09	0.1724
Pure error	242.67	2	121.33		
Total	21362.5	16			
R^2 = 0.8442					

[a] SS, sum of squares; DF, degrees of freedom; MS, mean squares; F_{ratio}: MS_{factor}/MS_{error}; P_{level}, probability level.
* Significant factor at α = 0.05.

Mindful of previous observations, the second phase involved a 2^2 central composite design. The new high (+), low (−) and central (0) levels for each factor were

E_{app} (+) = 0.4 V	$C_{substrate}$ (+) = 4.0 mM
E_{app} (−) = 0.2 V	$C_{substrate}$ (−) = 3.0 mM
E_{app} (0) = 0.3 V	$C_{substrate}$ (0) = 3.5 mM

On the basis of the results obtained by the different experimental designs, the optimum values of the experimental variables for the determination of As(V) using an AcP/SPCE biosensor were the following (Fig. 5):

E_{app} = +0.33 V	pH = 5.25	$C_{substrate}$ = 3.68 mM

Under these working conditions, the amperometric signal increased more than a hundred times in comparison with the signal obtained under the first conditions of the design.

Under these optimum conditions no amperometric response was obtained for 2-phospho-L-ascorbic acid when SPCEs were used. Likewise, no response was obtained for As(V) when no enzymatic modified SPCEs were utilized.

The kind of inhibitory effect of As(V) ions on the response of the acid phosphatase biosensors was also investigated following the method of Lineweaver–Burk [32,33]. Fig. 6 shows the competitive character of the registered inhibition process.

Once the optimal experimental conditions were found for the analysis of As(V) by means of AcP/SPCEs, a calibration was performed. A linear dependence was observed between ΔI and As(V) concentration in the range from 0.1 up to 1.3 μM. A least-median-squares regression (LMS) was used to detect the existence of anomalous points [25], which might have led to incorrect adjustments altering the sensitivity and the capability of detection.

A key feature of any analytical method is its capability of detection: the smallest concentration of the analyte that can be detected to a specified degree of certainty. The capability of detection, based on the variability of eight samples with a 0.1 μM concentration of As(V), was evaluated according to [34] and ISO 11843-2 [35]. At the chosen probability level of 5% ($\alpha = \beta = 0.05$), the capability of detection was 0.11 μM.

The precision of the developed method was calculated in terms of repeatability and reproducibility. In order to calculate the repeatability of the method, successive amperometric measurements with the same electrode surface were performed. The biosensors were conditioned at 4 °C for 1 h in a buffer solution pH 6 between experiments. Sets of five successive calibrations for As(V) were carried out yielding a relative standard deviation for their slopes of 7.11%. Likewise, the reproducibility of the

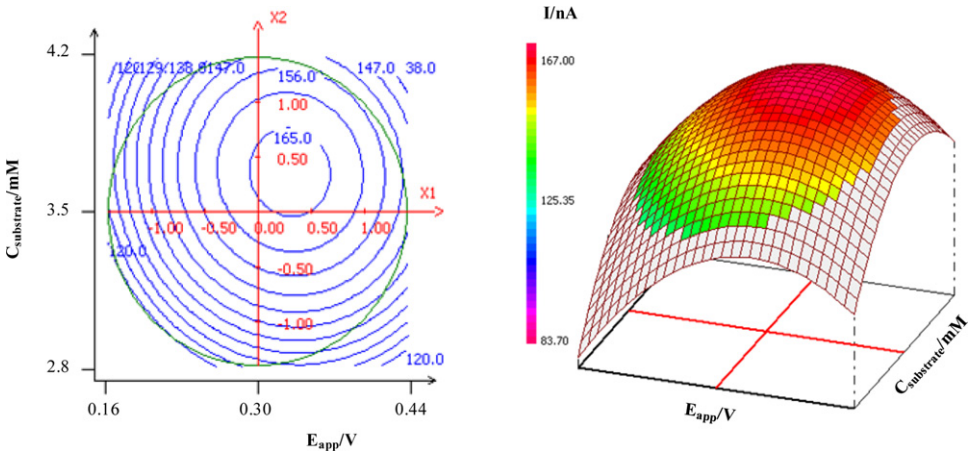


Fig. 5. Level curves and response surface plot for the 2^2 central composite design.

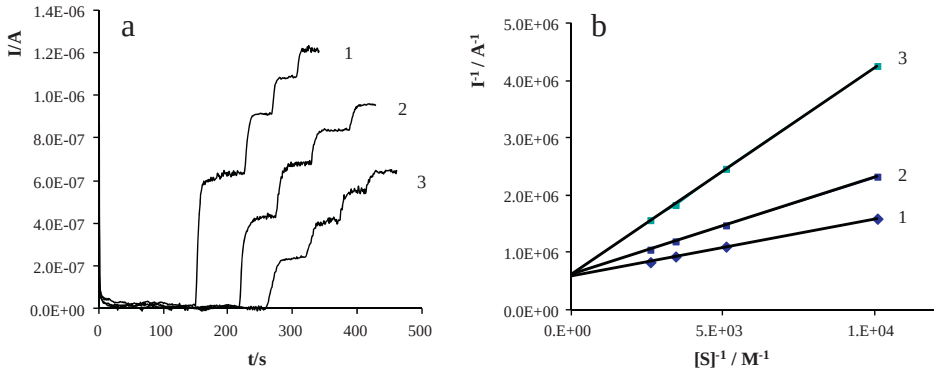


Fig. 6. Cronoamperometric curves (a) and Lineweaver Burk plots (b) for a AcP/SPCE: (1) without As(V), (2) with 20 μM As(V) and (3) with 200 μM. Acetate buffer pH 5.25 and 10 mM $MgCl_2$, E_{app} +0.33 V.

amperometric signal was checked using the slopes of five regressions carried out with different electrode surfaces. The RSD values obtained were 7.37%. These results suggest that the fabrication procedure of the AcP based biosensors is reliable, and they allow to obtain reproducible electroanalytical responses with different electrodes constructed in the same form.

The behavior of As(V) as an AcP inhibitor is not specific. The effect of the presence of some metal ions (As(III), Cd(II), Co(II), Cr(III), Cr(VI), Cu(II), Fe(III), Hg(II), Ni(II), Pb(II), Sb(III), Sb(V), Sn(II) and Zn(II)) was investigated.

In the case of Hg(II) and Pb(II) ions, instead of a decreased an increased of the analytical signal was observed for concentrations above 10 μM due to an enzyme activation effect.

Fe(III) ions apparently exerted an inhibitory effect in the AcP enzyme, giving also a decrease in the amperometric signal. However, this effect may be due to the formation of a stable complex with 2-phospho-L-ascorbic acid, which causes the consumption of the substrate and, consequently a decrease in the recorded signal. On the other hand, it is well known ascorbic acid reduces Cr(VI) to Cr(III) and Fe(III) to Fe(II). Therefore, in both cases the decrease of amperometric signals could be attributed to a substrate consumption by the interferent metal.

For the rest of the analyzed metal ions, no remarkable effects on the signal of the substrate were observed. Only when high concentrations (above 10^{-2} M) were added to the medium, a slight decrease in the signal was registered.

The developed disposable biosensor was successfully applied to the determination of As(V) in a ground water sample by standard addition. The ground water sample analyzed was obtained from a nearby source. Previously to the electrochemical determination, a preconcentration step was necessary. This step was carried out using a rotary evaporator at 40 °C and 72 mbar. Thus, the volume of the sample was reduced from 50 to 10 mL. Finally, additions of this preconcentrated sample were made into the electrochemical cell for the quantification of As(V) content.

The concentration found in the ground water sample was 2.90 ± 0.13 μM ($n = 3$, $\alpha = 0.05$). Good agreement was obtained between the concentration of As(V) found by the proposed method and the values obtained using ICP-MS as a reference technique (2.89 ± 0.02 μM ($n = 3$, $\alpha = 0.05$)).

Taking into account the source of the analyzed sample, it can be set that only As(V) is present. Thus, the results are definitely consistent with those provided by the ICP-MS.

5. Conclusions

The use of AcP-based inhibitor biosensors using SPCEs allows the selective and sensitive amperometric determination of As(V). The main experimental variables were optimized by means of central composite designs, founding values of +0.33 V, 3.68 mM and 5.25 for E_{app}, 2-phospho-L-ascorbic acid concentration and pH, respectively.

Comparing this study with previous described works, the proposed method offers several interesting advantages. In addition to its high reproducibility and repeatability, it should be highlighted the disposable character of SPCEs. Additionally, the construction

procedure described in this study resulted much easier and faster than those previously described in the literature.

Moreover, the proposed method allows the successful determination of As(V) in ground water samples.

Acknowledgments

The financial support made available by the *Junta de Castilla y León* (GR177) and the Spanish Ministry of Science and Innovation (TEC2009-12029) is gratefully acknowledged.

References

[1] P.L. Smedley, D.G. Kinniburgh, Appl. Geochem. 17 (2002) 517–568.
[2] E. Astolfi, A. Maccagno, J.C. García Fernández, Biol. Trace Elem. Res. 3 (1981) 133–143.
[3] K.G. Brown, T.L. Kuo, H.R. Guo, L.M. Ryan, C.O. Abernathy, Hum. Eco. Risk Assess. 6 (2000) 1055–1074.
[4] P.J. Mink, D.D. Alexander, L.M. Barraj, M.A. Kelsh, J.S. Tsuji, Regul. Toxicol. Pharmacol. 52 (2008) 299–310.
[5] I. Celik, L. Gallicchio, K. Boyd, T.K. Lam, G. Matanoski, X. Tao, M. Shiels, E. Hammond, L. Chen, K.A. Robinson, L.E. Caulfield, J.G. Herman, E. Guallar, A.J. Alberg, Environ. Res. 108 (2008) 48–55.
[6] Z. Gong, X. Lu, M. Ma, C. Watt, C. Leet, Talanta 58 (2002) 77–96.
[7] K. Jitmanee, M. Oshima, S. Motomizu, Talanta 66 (2005) 529–533.
[8] A.P. Packer, V.S.T. Ciminelli, Atom. Spectrosc. 26 (2005) 131–136.
[9] P.F. Jin, J. Ma, D. Zou, X.J. Wu, C.H. Sun, X. Hu, Chin. Pharm. J. 44 (2009) 548–551.
[10] P. Nisianakis, I. Giannenas, A. Gavriil, G. Kontopidis, I. Kyriazakis, Biol. Trace Elem. Res. 128 (2009) 62–71.
[11] H. Gramowska, I. Krzyzaniak, D. Baralkiewicz, R. Goldyn, Environ. Monit. Assess. 160 (2010) 479–490.
[12] I. Komorowicz, H. Gramowska, D. Baralkiewicz, J. Environ. Sci. Health A: Tox. Hazard Subst. Environ. Eng. 45 (2010) 348–354.
[13] P. Liang, L. Peng, P. Yan, Microchim. Acta 166 (2009) 47–52.
[14] J.Y. Cabon, N. Cabon, Anal. Chim. Acta 418 (2000) 19–31.
[15] J.Y. Cabon, N. Cabon, Fresenius J. Anal. Chem. 368 (2000) 484–489.
[16] I.B. Karadjova, L. Lampugnani, M. Onor, A.D' Ulivo, D.L. Tsalev, Spectrochim. Acta B: Atom. Spectrosc. 60 (2005) 816–823.
[17] V. Lenoble, V. Deluchat, B. Serpaud, J.C. Bollinger, Talanta 61 (2003) 267–276.
[18] B.R. Eggins, Biosensors: An Introduction, Wiley-Teubner, 1996.
[19] S. Cosnier, C. Mousty, X. Cui, X. Yang, S. Dong, Anal. Chem. 78 (2006) 4985–4989.
[20] M.A. Alonso-Lomillo, O. Domínguez-Renedo, L. Ferreira-Gonçalves, M.J. Arcos-Martínez, Biosens. Bioelectron. 25 (2010) 1333–1337.
[21] M.A. Alonso-Lomillo, O. Domínguez-Renedo, P. Matos, M.J. Arcos-Martínez, Anal. Chim. Acta 665 (2010) 26–31.
[22] S. Sanllorente-Méndez, O. Domínguez-Renedo, M.J. Arcos-Martínez, Sensors 10 (2010) 2119–2128.
[23] L. Asturias-Arribas, M.A. Alonso-Lomillo, O. Domínguez-Renedo, M.J. Arcos-Martínez, Anal. Chim. Acta 685 (2011) 15–20.
[24] D. Mathieu, J. Nony, R. Phan-Tan Luu, NEMRODW Version 2000, LPRAI, Marseille.
[25] P.J. Rousseeuw, A.M. Leroy, Robust Regression and Outlier Detection, John Wiley and Sons, New York, 1989.
[26] O. Domínguez, M.J. Arcos, Electrochem. Commun. 9 (2007) 820–826.
[27] J. Gonzalo-Ruiz, M.A. Alonso-Lomillo, F.J. Muñoz, Biosens. Bioelectron. 22 (2007) 1517–1521.
[28] S. Sanllorente-Méndez, O. Domínguez-Renedo, M.J. Arcos-Martínez, Electroanalysis 21 (2009) 635–639.
[29] E.J. Moore, M. Pravda, M.P. Kreuzer, G.G. Guilbault, Anal. Lett. 36 (2003) 303–315.
[30] D. Szydlowska, M. Campàs, J.L. Marty, M. Trojanowicz, Sens. Actuators B: Chem. 113 (2006) 787–796.
[31] A. Preechaworapun, Z. Dai, Y. Xiang, O. Chailapakul, J. Wang, Talanta 76 (2008) 424–431.
[32] M. Stoytcheva, V. Sharkova, Electroanalysis 14 (2002) 1007–1010.
[33] M. Stoytcheva, V. Sharkova, J.P. Magnin, Electroanalysis 10 (1998) 994–998.
[34] D.L. Massart, B.G.M. Vandeginste, L.M.C. Buydens, S.P. De Jong, J. Levi, S. Verdeke, Handbook of Chemometrics and Qualimetrics, Part A, Elsevier, Amsterdam, 1997.
[35] ISO 11843-2, Capability of Detection, ISO, Geneva, Switzerland, 2000.