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MASTER FINAL PROJECT

Master in Chemical Engineering

ELECTRODES MODIFICATION WITH SILVER NANOPARTICLES FOR THE DETECTION OF ARSENIC.

Report & Annex

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Resum

L'arsènic és un dels 10 productes químics de l'OMS (Organització Mundial de la Salut) de major preocupació per la salut pública. El límit actual recomanat d'arsènic a l'aigua potable és de 10 ppb. Les tècniques habituals per la seva quantificació són cares i utilitzen instruments grans i voluminosos que només són aptes per al seu ús en laboratori. En canvi, les tècniques voltamperomètriques són més simples, de baix cost i permeten la utilització d'equips portàtils capaços de determinar elements traça. Aquest és el cas dels elèctrodes *screen printed* (SPE). A més a més, la composició de la seva superfície pot ser fàcilment modificada amb l'addició de nanopartícules per millorar la sensibilitat.

En aquest sentit, es va proposar detectar arsènic en solució mitjançant voltamperometria i SPEs modificats amb nanopartícules de plata, les quals es poden obtenir per diferents tipus de síntesi. En aquest treball s'han estudiat dos tipus de nanopartícules (NPs): de via sintètica de diferents formes i mides (llavors i nanoprismes de plata) i també NPs de síntesi respectuosa amb el medi ambient o "verdes". Tots els tipus es van caracteritzar microscòpicament i es van utilitzar en la modificació de SPEs comercials de nanofibres de carboni.

La determinació d'arsènic es va realitzar mitjançant voltamperometria de redissolució anòdica en el mode de pulsos diferencials (DPASV) i es van optimitzar els paràmetres experimentals. El millor resultat es va aconseguir amb les NPs sintètiques i més concretament, amb les llavors de plata, aconseguint un límit de detecció de 1,66 ppb per un rang lineal de fins 50 ppb. Finalment, es va aplicar a una mostra real (aigua de l'aixeta "spiked") , de la qual s'ha pogut determinar la seva concentració amb una desviació d'un 1%.

Resumen

El arsénico es uno de los 10 productos químicos de la OMS (Organización Mundial de la Salud) de mayor preocupación para la salud pública. El límite actual recomendado de arsénico en el agua potable es de 10 ppb. Las técnicas habituales para su cuantificación son caras y utilizan instrumentos grandes y voluminosos que sólo son aptos para su uso en laboratorio. En cambio, las técnicas voltamperométricas son más simples, de bajo coste y permiten la utilización de equipos portátiles capaces de determinar elementos traza. Este es el caso de los electrodos *screen printed* (SPE). Además, la composición de su superficie puede ser fácilmente modificada con la adición de nanopartículas para mejorar la sensibilidad.

En este sentido, se propuso detectar arsénico en solución mediante voltamperometría y SPEs modificados con nanopartículas de plata, las cuales se pueden obtener por diferentes tipos de síntesi. En este trabajo, se estudiaron dos tipos de nanopartículas (NPs): de vía sintética de diferentes formas y tamaños (semillas y nanoprismas de plata) y NPs de síntesi respetuosa con el medio ambiente o “verdes”. Ambos tipos se caracterizaron microscópicamente y se utilizaron en la modificación de SPEs comerciales de nanofibras de carbono.

La determinación de arsénico se realizó mediante voltamperometría de redisolución anódica en el modo de pulsos diferenciales (DPASV) y se optimizaron los parámetros experimentales. El mejor resultado se logró con las NPs sintéticas y más concretamente, con las semillas de plata, logrando un límite de detección de 1,35 ppb para un rango lineal de concentración de hasta 50 ppb. Finalmente, se aplicó a una muestra real (agua del grifo "spiked"), de la que se ha podido determinar su concentración con una desviación de un 1%.

Abstract

Arsenic is one of the 10 chemicals of the World Health Organization (WHO) of major public health concern. The current recommended limit of arsenic in drinking water is 10 ppb. The usual techniques for quantification are expensive and use large and bulky instruments that are only suitable for use in the laboratory. On the other hand, voltammetry techniques are simpler, inexpensive and allow the use of portable equipment capable of determining trace elements. This is the case of screen printed electrodes (SPE). In addition, the surface composition thereof can be easily modified with the addition of nanoparticles to improve sensitivity.

In this sense, it was proposed to detect arsenic in solution by means of voltammetry and SPEs modified with silver nanoparticles, which can be obtained by synthetic route and by "green" route. In this work, two types of nanoparticles (NPs) were studied: synthetic pathway NPs of different shapes and sizes (seeds and nanoprisms) and also environmentally friendly NPs or “green” source NPs. Both types were characterized microscopically and were used in the modification of commercial SPEs of carbon nanofibers.

The determination of arsenic was performed by differential pulse anodic stripping voltammetry (DPASV) and the experimental parameters were optimized. The best result was achieved with synthetic NPs and more specifically with silver seeds, achieving a detection limit of 1,66 ppb for a linear concentration range of up to 50 ppb. Finally, it was applied to a real sample ("spiked” tap water), from which it was possible to determine its concentration with a deviation of 1%.

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Glossary

AE	Auxiliary electrode
AgNPs	Silver nanoparticles
AgNPs-SPCNFE	SPCNFE modified with silver nanoparticles
AgNPs-SPE	SPE modified with silver nanoparticles
ASV	Anodic stripping voltammetry
DME	Dropping mercury electrode
DPASV	Differential pulse anodic stripping voltammetry
DPV	Differential pulse voltammetry
E_d	Deposition potential
E_i	Initial potential
I	Current
IARC	International Agency for Research on Cancer
ICP	Inductively coupled plasma
LOD	Limit of detection
LOQ	Limit of quantification
NPs	Nanoparticles
ppb	Parts per billion
ppm	Parts per million
RE	Reference electrode
SEM	Scanning electron microscope
SPCNFE	Carbon nanofiber modified screen-printed electrode
SPE	Screen printed electrode
t_d	Deposition time
TEM	Transmission Electron Microscopy
U	Potential
WE	Working electrode
WHO	World Health Organization

Índex

1. PREFACE

1.1. Origin of the project

The present Master Final Project is a contribution to the research project "Síntesis verde de nanopartículas metálicas a partir de aguas ácidas de mina y extractos de residuos agroalimentarios" funded by Ministerio de Economía y Competitividad, Madrid, and FEDER funds, EU, 2016-2018. Project CTM2015-68859-C2-2-R (MINECO/FEDER).

1.2. Motivation

The main motivation of this project is to be able to quantify the concentration of arsenic, a very toxic heavy metal, by means of voltammetry, an electrochemical technique which is fast and economical. It is intended to improve the detection limits of the metal using nanoparticles obtained with synthetic and green sources.

2. INTRODUCTION

2.1. Arsenic properties

Arsenic (from the Greek word "arsenikos" and the Latin word "arsenicum," meaning yellow orpiment) is a chemical element of the periodic table that belongs to the group of metalloids, also called semimetals, and can be found in various forms, although rarely in the solid state.

Since its isolation in 1250 A.D. by Albertus Magnus, this element has been a center of controversy in human history. It has been used in medicine and in various fields i.e. agriculture, livestock, electronics, industry and metallurgy (1). Some examples of the current applications are listed above:

- Preservative of wood (copper and chromium arsenate), use which, according to some estimates, represents about 70% of the world consumption of arsenic.
- The optoelectronic compound gallium arsenide is an important semiconductor material used in faster and more expensive integrated circuits than silicon. It is also used in the construction of LED and laser diodes.
- Additive in lead and brass alloys (for example, in car batteries and ammunition).
- Insecticide (lead arsenate), herbicides (sodium arsenite) and poisons: In the early twentieth century were used inorganic compounds but their use has virtually disappeared for the benefit of organic compounds (methyl derivatives).
- Arsenic disulfide is used as a pigment and in pyrotechnics.
- Coloring agent in the manufacture of glass (arsenic trioxide).

However, it is now well recognized that consumption of arsenic, even at low levels, leads to carcinogenesis and thus, some applications are declining in their use:

- Historically, arsenic has also been used for therapeutic purposes practically abandoned by Western medicine.
- The agricultural use of arsenic is declining, as a fertilizing element in the form of a rich primary mineral.
- Throughout history, arsenic and its compounds have been used for homicidal purposes, mainly in the form of arsenious anhydride (white powder, tasteless and odorless called king of poisons).
- Manufacture of insecticides, herbicides, rodenticides, fungicides, etc.

In 2014, the world total production of As_2O_3 was 36.400 of tons (2).

2.2. Arsenic distribution on the environment and sources

Arsenic is common in the Earth's atmosphere, in rocks and soils, in the hydrosphere and biosphere and comprises about 1,5 ppm (0,00015%) of the Earth's crust, being the 53rd most abundant element. Therefore, it is widely distributed in surface water, groundwater, and drinking water. It is brought to the environment through a combination of processes such as:

- Natural factors such as weathering since this element occurs in trace amounts in most rocks as well as in soil, water, and atmospheric dust.; biological activity, volcanic emissions…
- Anthropogenic such as mining, use of fossil fuels, use of pesticides, herbicides, etc.

Once released into the environment, arsenic compounds reach water sources, such as rivers and groundwater systems, and subsequently food sources. Arsenic-contaminated soil, sediment, and sludge are the major sources of arsenic in the food chain, surface water, groundwater, and drinking water. Arsenic concentrations in non-contaminated soils are typically below 10 mg/kg while in contaminated soils they can be as high as 30.000 mg/kg (3).

Concentrations in water may be elevated in areas with volcanic rock and sulfide mineral deposits; in areas containing natural sources, where levels as high as 12 mg/l have been reported; near anthropogenic sources, such as mining and agrochemical manufacture; and in geothermal waters (average 500 µg/l or 500 ppb, maximum 25 mg/l or 25 ppm) (4).

It is also found in sea water at a level of about 2 µg/kg, in groundwater sometimes at concentrations exceeding 21 mg/kg and it also occurs in the atmosphere through burning of fossil fuels and smelting of non-ferrous ores, as well as naturally through volcanism and from the oceans by bubble bursting (5).

Terrestrial plants and freshwater fish contain arsenic at levels of 0,05-0,2 mg/kg and sometimes at higher concentrations when anthropogenic contamination has occurred. On the other hand, marine animals and algae, because of biotransformation and accumulation, contain high concentrations of arsenic, typically in the range 1-100 mg/kg (5).

In the soil environment, arsenic is present mostly as the inorganic species (arsenate As(V) and arsenite As(III)) but also as As(0) and As(-III)). Importantly, the different forms of As show various hazard levels. The inorganic arsenic species (such as those found in water) can be more toxic than the organic As species (such as those found in seafood). Furthermore, As(III) is reported to be more toxic and soluble than As(V) (6).

In water, it is most likely to be present as arsenate if the water is oxygenated. However, under reducing conditions (<200 mV), it is more likely to be present as arsenite.

2.3. Toxicity of arsenic and exposure

Arsenic is one of WHO's (World Health Organization) 10 chemicals of major public health concern. The current recommended limit of arsenic in drinking-water is 10 μg/l or 10 ppb.

The immediate symptoms of acute arsenic poisoning include vomiting, abdominal pain and diarrhea. These are followed by numbness and tingling of the extremities, muscle cramping and death, in extreme cases.

The first symptoms of long-term exposure to high levels of inorganic arsenic (e.g. through drinking-water and food) are usually observed in the skin, and include pigmentation changes, skin lesions and hard patches on the palms and soles of the feet (hyperkeratosis). These occur after a minimum exposure of approximately five years and may be a precursor to skin cancer. In addition to skin cancer, long-term exposure to arsenic may also cause cancers of the bladder and lungs. The International Agency for Research on Cancer (IARC) has classified arsenic and arsenic compounds as carcinogenic to humans. Other adverse health effects that may be associated with long-term ingestion of inorganic arsenic include developmental effects, neurotoxicity, diabetes, pulmonary disease and cardiovascular disease. Arsenic-induced myocardial infarction, in particular, can be a significant cause of excess mortality. Arsenic is also associated with adverse pregnancy outcomes and infant mortality, with impacts on child health, and there is some evidence of negative impacts on cognitive development (4).

In short, chronic arsenic toxicity produces various systemic manifestations over and above skin lesions. Table 1 reports clinical features of 156 cases of arsenicosis caused by chronically drinking arsenic-contaminated water in West Bengal, India (7).

Table 1. Clinical features of 156 cases of chronic arsenicosis studied in West Bengal, India. (7)

SYMPTOMS	% OF CASES	SYMPTOMS	% OF CASES
Weakness	70,5	**Dyspnea**	23,7
Headache	20,5	**Paresthesia**	47,4
Burning of the eyes	44,2	**Pigmentation**	100
Nausea	10,9	**Keratosis**	61,5
Pain in the abdomen	38,4	**Anemia**	47,4
- **Epigastric**	25	**Hepatomegaly**	76,9
- **Paraumbilical**	13,4	**Splenomegaly**	31,4
Diarrhea	32,6	**Ascites**	3
Cough	57	**Pedal edema**	11,5
- **With expectoration**	33,9	**Sign of lung disease**	28,8
- **Without expectoration**	23,1	**Sign of polyneuropathy**	13,4
Hemoptysis	5,1		

Except for individuals who are occupationally exposed to arsenic, the most important route of exposure is through the oral intake of food and drinking-water, including beverages made from drinking-water and using contaminated water in food preparation. Other ways to be exposed are by irrigation of food crops, industrial processes and smoking tobacco.

The mean daily intake of arsenic from drinking-water will generally be less than 10 µg; however, in those areas in which drinking-water contains elevated concentrations of arsenic, this source will make an increasingly significant contribution to the total intake of inorganic arsenic as the concentration of arsenic in drinking-water increases. As the estimated daily intake of arsenic from food in preliminary studies of diets in North America is 12–14 µg of inorganic arsenic, consumption of 2 liters of drinking-water containing 10 µg/l would make drinking-water the dominant source of intake. In circumstances where rice, soups or similar dishes are a staple part of the diet, the drinking-water contribution through preparation of food will be even greater. Fish, shellfish, meat, poultry, dairy products and cereals can also be dietary sources of arsenic, although exposure from these foods is generally much lower compared to exposure through contaminated groundwater. In seafood, arsenic is mainly found in its less toxic organic form. The estimated intake from air is generally less than 1 µg (4).

Therefore, the greatest threat to public health from arsenic originates from contaminated groundwater. Inorganic arsenic is naturally present at high levels in the groundwater of a number of countries, including Argentina, Bangladesh, Chile, China, India, Mexico, and the United States of America (4).

2.4. Determination of Arsenic

As mentioned before, arsenic is one of WHO's 10 chemicals of major public concern so the importance of this heavy metal determination and monitoring is a well-recognized fact that is emphasized by the extensive studies carried out in this area. A vast detection methods have been developed for determination of arsenic, such as atomic fluorescence spectrometry (AFS), hydride generation atomic absorption spectrometry (HGAAS), inductively coupled plasma-mass spectrometry (ICP-MS), inductively coupled plasma atomic emission spectrometry (ICP-AES), graphite furnace atomic absorption (GFAA) and high-performance liquid chromatography-inductively coupled plasma mass spectrometry (HPLC-ICPMS).

The USEPA (US Environmental Protection Agency) has elaborated a document reviewing the science and technologies for monitoring arsenic in the environment (USEPA 2004). The methods approved by USEPA include ICP-MS, ICP-AES, GFAA, and HGAAS, all of which may be characterized by limit of detection (LOD) ranging from 0,5 to 50 ppb (8). Table 2 shows the different methods with the LODs (9). The choice of an adequate analytical method is dictated by the purpose of the analysis,

the level of analyte's concentration in concrete matrix, and the type of sample in which analyte is present.

Table 2. Analytical techniques for the analysis of arsenic in water (9).

TECHNIQUE	LOD (µg/l)
ICP-MS	1,4
ICP-AES	8
GFAA	0,5
HGAAS	0,5
HPLC-ICPMS	1
AFS	0,67

However, these methods use large and bulky instruments that are only suitable for laboratory use. Besides, the operational and instrumental costs are really expensive as well as their maintenance and are also time-consuming. Because of these factors, these techniques are impractical for on-site screening and not useful for routine monitoring of large numbers of samples.

Electroanalytical methods offers several attractive advantages over traditional analytical methods such as relative simplicity, low costs and portable field-based equipment able to determine trace elements. Furthermore, it also provides high sensitivity and low detection limits, which is important for the determination of the low concentrations of arsenic in waters.

Electrochemical methods are based on the measurement of electrical signals associated with molecular properties or interfacial processes of chemical species. The different types of electrochemical methods are listed in the Figure 1. Voltammetric methods are among the most used electrochemical techniques for the determination of heavy metals in natural samples.

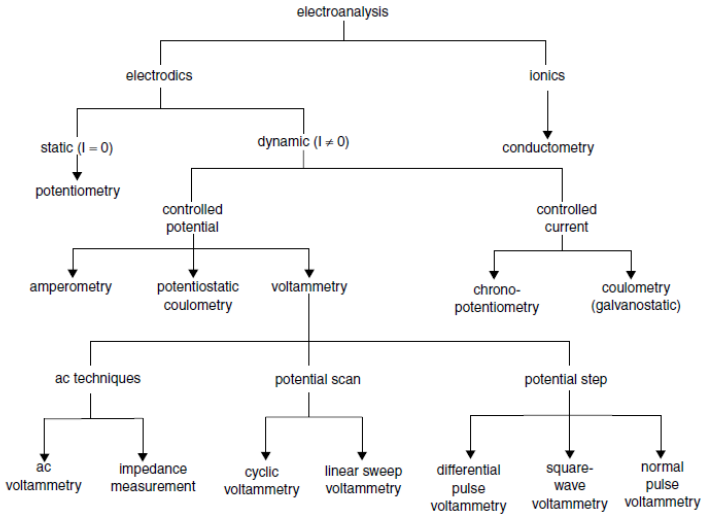


Figure 1. Scheme of the different types of electrochemical methods.

2.5. Voltammetry

Voltammetry is one of the main electroanalytical techniques. Voltammetric techniques involve the application of a potential (U) to an electrode and the monitoring of the resulting current (I) flowing through the electrochemical cell. In many cases the applied potential is varied or the current is monitored over a period of time (t). Thus, all voltammetric techniques can be described as some function of U, I, and t. The applied potential forces a change in the concentration of an electroactive species at the electrode surface by electrochemically reducing or oxidizing it.

The analytical advantages of the voltammetric techniques include excellent sensitivity with a very large useful linear concentration range for both inorganic and organic species (10^{-12} to 10^{-1} M), a large number of useful solvents and electrolytes, a wide range of temperatures, rapid analysis times (seconds), simultaneous determination of several analytes, the ability to determine kinetic and mechanistic parameters, a well-developed theory and thus the ability to reasonably estimate the values of unknown parameters, and the ease with which different potential waveforms can be generated and small currents measured.

Analytical chemists routinely use voltammetric techniques for the quantitative determination of a variety of dissolved inorganic and organic substances. Also it is useful for fundamental studies of oxidation and reduction processes in various media, adsorption processes on surfaces, electron transfer and reaction mechanisms, kinetics of electron transfer processes, and transport, speciation, and thermodynamic properties of solvated species. Voltammetric methods are also applied to the determination of compounds of pharmaceutical interest and, when coupled with HPLC, they are effective tools for the analysis of complex mixtures (10).

2.5.1. Voltammetry mechanism

The electrochemical cell, where the voltammetric experiment is carried out, consists of a working electrode, a reference electrode, and usually a counter (auxiliary) electrode. In general, an electrode provides the interface across which a charge can be transferred or its effects felt. The reduction or oxidation of a substance at the surface of a working electrode, at the appropriate applied potential, results in the mass transport of new material to the electrode surface and the generation of a current. As the potential of the electrode becomes more negative, it becomes more strongly reducing. Conversely, as the potential becomes more positive, it becomes more strongly oxidizing. Therefore, the redox reaction taking place on the electrode can be controlled by controlling the electrode potential.

The current, on the other hand, is simply a measure of electron flow and it is due to electron transfer which takes place when an oxidation or reduction occurs on the electrode surface. This type of current is termed Faradaic and is proportional to concentration. The current due to a reduction

(cathodic current) is, by convention, assigned a positive sign. The current due to an oxidation (anodic current) is assigned a negative sign.

An illustrative example of how voltammetry works is represented in Figure 2, which shows details for a solution which contains Pb and Cd ions.

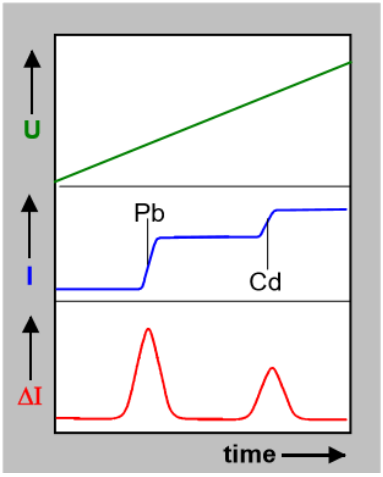


Figure 2. Example plots of voltammetry that shows details for a solution which contains Pb and Cd ions (11).

The different curves represents:

1. Voltage ramp which is applied on the working electrode.
2. Two waves appear at different potentials, one for Pb, another for Cd.
3. Mathematical differentiation of the original curve.

The resulting plot of current versus applied potential is called a voltammogram (Figure 3) and it is the electrochemical equivalent of a spectrum in spectroscopy, providing quantitave and qualitative information about the species involved in the oxidation or reduction reaction since the potential of the reduction is characteristic of each ion.

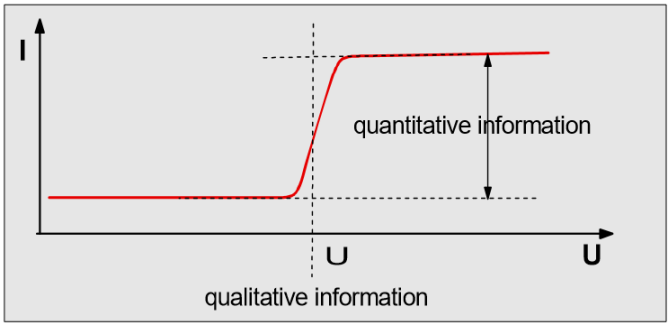


Figure 3. Example voltammogram, current versus potential (11).

2.5.2. Instrumentation

The basic components of a modern electroanalytical system for voltammetry are a potentiostat, computer, and the electrochemical cell. Figure 4 represents a basic set up for voltammetry measurements.

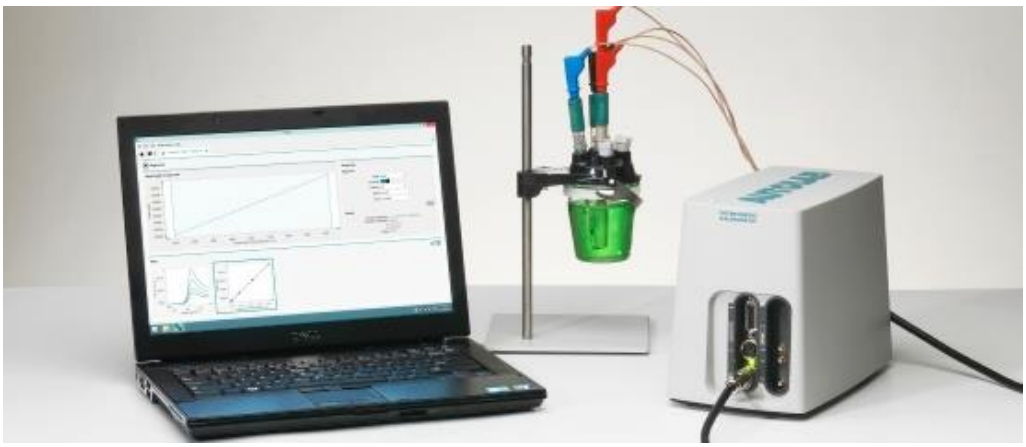


Figure 4. Set-up for voltammetric measurements.

2.5.2.1. The potentiostat

A potentiostat is a device that controls the potential applied to a working electrode (WE), relative to a reference electrode (RE), and measures the current flowing between the working electrode and a third electrode, called the auxiliary electrode (AE). The most widely used potentiostats today are assembled from discrete integrated-circuit operational amplifiers (OAs), which are circuit components having dual inputs of very high impedance. One of the inputs inverts the polarity of the applied signal (the inverting input, -), while the other is noninverting (+) with respect to the input polarity.

2.5.2.2. The Electrodes and Cell

2.5.2.2.1 Cell

A typical electrochemical cell consists of the sample dissolved in a solvent, an ionic electrolyte, and three (or sometimes two) electrodes (Figure 5). Cells (that is, sample holders) come in a variety of sizes, shapes, and materials. The type used depends on the amount and type of sample, the technique, and the analytical data to be obtained. The material of the cell (glass, Teflon, polyethylene) is selected to minimize reaction with the sample (10).

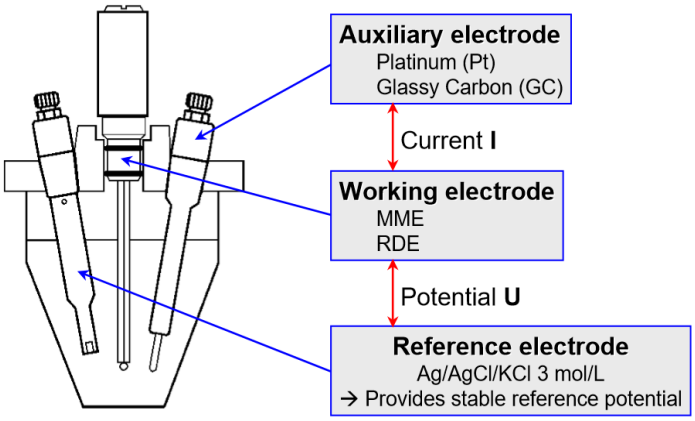


Figure 5. Scheme of a typical electrochemical cell. (11).

2.5.2.2.2 Reference Electrodes

The reference electrode should provide a reversible half-reaction with Nernstian behavior, be constant over time, and be easy to assemble and maintain. The most commonly used reference electrodes for aqueous solutions are the calomel electrode, with potential determined by the reaction:

$$Hg_2Cl_2(s) + 2e^- \rightarrow 2Hg(l) + 2Cl^- \quad \textbf{(Eq. 1)}$$

and the silver/silver chloride electrode (Ag/AgCl), with potential determined by the reaction:

$$AgCl(s) + e^- \rightarrow Ag(s) + Cl \quad \textbf{(Eq. 2)}$$

2.5.2.2.3 Counter Electrodes / Auxiliary Electrode

The auxiliary electrode passes all the current needed to balance the current observed at the WE. To achieve this current, the auxiliary will often swing to extreme potentials at the edges of the solvent window, where it oxidizes or reduces the solvent or supporting electrolyte. Most often the counter electrode consists of a thin Pt wire, although Au and sometimes graphite have also been used.

2.5.2.2.4 Working Electrodes

The WE, which makes contact with the analyte, must apply the desired potential in a controlled way and facilitate the transfer of charge to and from the analyte. The WEs are of various geometries and materials, ranging from small Hg drops to flat Pt disks. Mercury is useful because it displays a wide negative potential range (because it is difficult to reduce hydrogen ion or water at the mercury surface), its surface is readily regenerated by producing a new drop or film, and many metal ions

can be reversibly reduced into it. Other commonly used electrode materials are gold, platinum, and glassy carbon.

2.5.3. Stripping voltammetry

Voltammetric stripping techniques provide accurate measurements of low metal ions concentrations at the ppb levels, especially heavy metals in water, with rapid analysis times and low-cost instrumentation. The techniques usually consist of a pre-concentration of the metals in the electrode surface, followed by a potential sweep to dissolve pre-concentrated species of interest, making the quantification of them. The potential sweep to obtain the stripping scan can be performed by various techniques such as differential pulse, square wave, linear sweep or staircase. The two most commonly used are the differential pulse and square wave, due to their advantages (12).

Depending on the nature of the analyte, different variations of the methods of accumulation (electrolytic or adsorptive) and methods of detection (voltammetry or potentiometry) are used, for example, anodic stripping voltammetry (ASV), cathodic stripping voltammetry (CSV), and adsorptive stripping voltammetry (AdSV). Among these, the most common is ASV.

The stripping techniques have lower detection limit than any of the commonly used electrochemical techniques, and the preparation of the sample is simple, in addition the sensitivity and the selectivity are excellent.

The response due to a given analyte in the stripping step is proportional to the concentration of that analyte in the solution. The conversion of the response to a concentration can be achieved using a calibration curve, but the method of standard additions is generally used. In this method, a known amount of the analyte is added to the solution and the experiment is repeated.

The procedure consists of three main steps:

- Step of pre-concentration: the analyte species in the sample solution is concentrated onto or into a working electrode by electrolysis with a constant deposition potential (E_d). This step provides different modes for pre-concentration of the analyte on the working electrode, which can be electrochemically (with or without potential stimulus) and by adsorptive. The concentration of the analyte on or in the electrode is therefore much higher than the concentration in the solution (up to a 1000-fold increase). To achieve reproducible results is necessary control the hydrodynamic parameters (pre-concentration time, stirring, temperature, electrode area and initial potential applied (E_i). The pre-concentration allows an increase of sensitivity of other 2 or 3 orders of magnitude, making it feasible to operate with analyte concentrations 10^{-10} M or even lower, sensitivities comparable with others techniques not electro-analytical characterized by high sensitivity (13).

- Step of Resting: After a time perfectly measured (usually lasts for 5 to 30 s), stops electrolysis and stirring so there is no convection, but remains constant initial potential. This step allows the analyte to homogeneously distribute on the electrode.
- Step of Stripping: During this step, the deposited analyte is determined by a voltammetric procedure by stripping itself, through a potential sweep in the opposite direction to the initial. (14).

2.5.4. Anodic stripping voltammetry

ASV is most widely used for trace metal determination and has a practical detection limit in the part per-trillion range (10). In this case, the analyte of interest is electroplated on the working electrode during a cathodic deposition step, and oxidized from the electrode during the anodic stripping step, which can be either linear (LSASV), square wave (SWASV), or pulse (DPASV).

The accumulation step covers the reducing of metal cations at a constant potential, called deposition potential (E_d), for several minutes, especially assisted by convection, forming a composite or alloy (bismuth electrodes and mercury). The resulting concentration of the element to be detected in the electrode is substantially greater than in the solution analyzed due to the volume of the electrode is much smaller in comparison with the volume of solution. For example, in the case of a mercury electrode, the metal ions reach the mercury electrode by diffusion and convection, where they are reduced and concentrated as amalgams:

$$M^{n+} + ne^{-} + Hg \rightarrow M(Hg) \qquad \textbf{(Eq. 3)}$$

The transport by convection is achieved by the agitation of the solution. The duration of the deposition step (t_d) is selected as a function of the concentration level of the metal ion to be determined.

During the resting step, the stirring is stopped, causing a drop in the cathodic current due to the lack of convection. This period serves for dissolved analyte to distribute well in the drop of mercury and lasts for 5 to 30 seconds.

After to finish stirring, the potential is changed to a more positive potential (anodic potential) by means of a linear sweep voltammetry, differential pulse or square wave, which results in stripping (oxidation) of the metal, the amalgam or alloy, returning again to the solution and recording a current peak due to it gives off electrons. The current peak height or area reflecting the detected concentration of the material in the electrode is proportional to the amount of material dissolved in the solution as long as they remain appropriate experimental parameters such as the area of the electrode, potential pre-concentration time, time and potential of deposition, conditions of stirring, temperature, etc. (14)

In the mercury electrode, the amalgamated metals are stripped out from the electrode producing characteristic peaks:

$$M(Hg) \rightarrow M^{n+} + ne^{-} + Hg \quad \textbf{(Eq. 4)}$$

With this ASV approach, about 15 amalgam-forming metals can be determined, including Tl, Cd, Zn, Cu, Bi, In and Ga. The trace analysis of other metal ions (Hg, Au, As, Se) can be performed after their electrolytic deposition as a metal film on bare solid electrodes made from carbon or gold (15).

ASV has been widely recognized as a powerful technique for arsenic ion detection, owing to its remarkable sensitivity. Moreover, these techniques can also be readily carried out with cost-effective and easy-to-use instrumentation. According to literature for electrochemical As(III) detection, the gold electrode is the most suitable one, due to the stable Au-As intermetallic compounds formed during the deposition step. There are many papers where the arsenic detection is carried out using bulk, film gold electrodes and even with Au nanoparticles (AuNPs) modified electrodes (16).

2.5.5. Differential pulse voltammetry

The differential pulse voltammetry, also called differential pulse polarography (DPV, DPP), is a type of technique for the potential sweep in the stripping step, that combines a linear voltage ramp with pulses of a fixed magnitude (magnitude 5-250 mV) which are repeated once during each drop lifetime and last about 60 ms. The current is measured twice: once before applying the pulse (I_a) and once during the last 17 ms of the pulse (I_b). The first current is instrumentally subtracted from the second current. The differential pulse voltammogram is thus a plot of current difference (I_b-I_a) versus applied potential. The use of the pulse minimizes the effect of charging current.

The measurement of current difference gives the differential pulse voltammogram a peak shape (Figure 6). The peak height (I_p) is directly proportional to the analyte concentration in solution. Moreover, the pulse amplitude also increases.

If the stripping step in an ASV is differential pulse, the method is called differential pulse anodic stripping voltammetry (DPASV).

Forsberg et al. investigated in detail the determination of arsenic by ASV and differential pulse anodic stripping voltammetry (DPASV) at various electrode materials (HMDE, Pt and Au). This experiments showed that ASV proved to be an exceedingly sensitive technique as for the determination arsenic, while the use of DPASV greatly shortened deposition times (17).

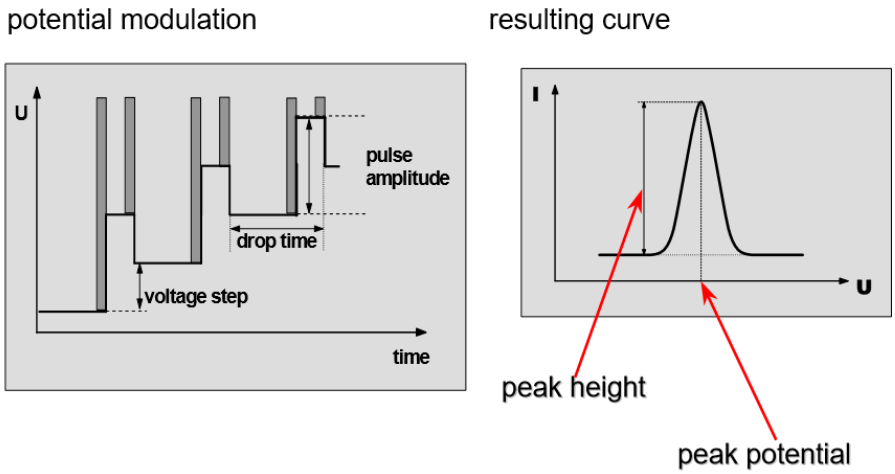


Figure 6. Examples of differential pulse voltammograms (11).

2.6. Working electrodes

The selection of a working electrode material for voltammetric measurements is critical to obtain a successful and reproducible response. Two important factors should be considered for the selection of the working electrode. Firstly, the material should exhibit favorable redox behavior with the analyte, ideally fast, reproducible electron transfer without electrode fouling. Secondly, the *potential window* over which the electrode performs in a given electrolyte solution should be as wide as possible to allow for the greatest degree of analyte characterization. Additional considerations include the cost of the material, its ability to be machined or formed into useful geometries, the ease of surface renewal following a measurement, and toxicity (18).

The most commonly used working electrode materials are platinum, gold, carbon, and mercury. Table 3 lists summarizes the advantages and limitations of each material as WE.

Table 3. Table of the most commonly used electrode materials (18).

Material	Advantages	Drawbacks
Hg	✓ excellent potential window in the cathodic direction ✓ it has a high reproducibility ✓ easy to " refresh"	× toxic × limited in the anodic direction
Pt	✓ good electrochemical inertness ✓ ease of fabrication into many forms	× high cost × leads to hydrogen evolution in presence of water or acid × limited
Au	✓ larger cathodic potential range ✓ useful for constructing self-assembled monolayers	× expensive oxidation of its surface

Carbon	✓ allow scans to more negative potentials than platinum ✓ good anodic potential windows ✓ good cathodic potential range ✓ many types and configurations	× quality varies greatly × hard to shape

Mercury has historically been the most used electrode material, primarily as a spherical drop formed at the end of a glass capillary through which the liquid metal is allowed to flow. There are different types of electrodes with it: the dropping mercury electrode (DME), the static mercury drop electrode (SMDE) and the hanging mercury drop electrode (HMDE). Among these, the DME is the most used as it displays an excellent potential window in the cathodic direction and it has a high reproducibility. In the DME, drops are formed and fall off repeatedly during a potential scan, being replaced by a "fresh" electrode every second or so (18).

However, it has a several drawbacks as it is severely limited in the anodic direction by its ease of oxidation. Furthermore, the toxicity and environmental hazard of mercury has led to a limited use these days. This problems have stimulated the development of other types of electrodes such as bismuth and carbon electrodes which advantages are their low background current, wide potential range, chemical inertness and suitability for various sensing and detection applications.

Various electrode materials, such as HMDE, Pt and Au have been used in the determination of arsenic through DPASV. The results were Au provides a more sensitive response toward arsenic oxidation than the other electrode materials studied and has a higher hydrogen overvoltage than platinum (17).

2.6.1. Screen printed electrodes

An alternative to the use of the mercury electrode on the determination of metal ions are the screen printed electrodes (SPE). Their use in voltammetry devices is well recognized and have been reported for the detection of heavy metals such as copper, lead, cadmium, mercury, antimony, arsenic and more (19).

SPE are miniaturized planar devices with plastic substrates that are coated with layers of electroconductive and insulating inks at a controlled thickness. The main advantage of SPE over conventional WE is the opportunity to apply electrochemical techniques for environmental analyses outside a centralized laboratory because of the possibility of connecting them to portable instrumentation. Thus, the sample can be analyzed *in-situ*.

Furthermore, the advent of screen printing technology has made it possible to mass production of SPE, making them a low cost device and thus, disposable. There are different commercial distributors of SPE such as DropSens. However, there is also the possibility to manufacture it in laboratories with screen printing machines.

SPE usually include a three electrode configuration (working, auxiliary and reference electrodes) printed on the same strip which, together with their miniaturized size and their possibility to be connected to portable instrumentation, makes them more suitable for *in-situ* analysis (Figure 7).

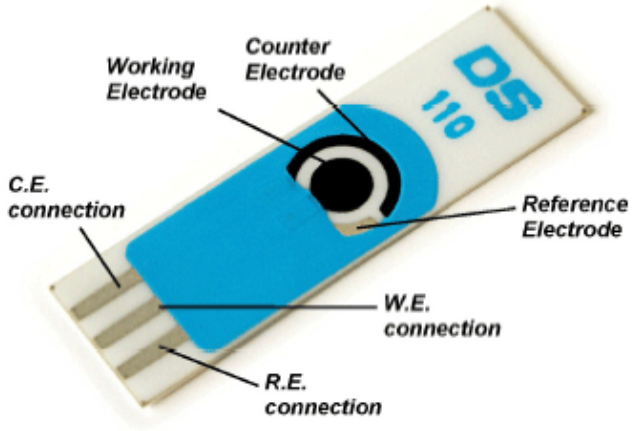


Figure 7. DropSens SPE's configuration**.**

The great versatility of screen-printed electrodes resides in their wide range of possible modifications to achieve a variety of improvements in the detection of different pollutants. Its composition surface can be easily modified with the addition of different substances in the printing process such as metals, enzymes, polymers, complexing agents, etc. and more recently with nanoparticles. In particular, most studies of heavy-metal determination using SPE show that mercury, gold, silver, bismuth or other materials modified on the surface of SPE can improve selectivity or sensitivity (20).

The most appropriate material for the electrode's surface is carbon- based nanomaterial due to its low cost and because it enhances the effective surface and improves the electron-transfer kinetics. In particular, it has been reported that sensors with better analytical performance can be obtained if a carbon nanofiber modified screen-printed electrode (SPCNFE) is used as electrode substrate, since carbon nanofibers are easier to functionalize and simultaneously provide mechanical and electrical property enhancements (21).

In the literature, several SPE modifications can be found for the determination of As(III) with limit of detections (LOD) lower than 10 ppb, which is the limit proposed by WHO for water consumption. Some successful modifications can be shown in the Table 4, with proven applications. Notice that there is almost no modification for the direct detection for As(V). Some papers affirms that it is electrochemical neutral (22),(19) so the method to quantification is indirect: determination of As(III), followed by reduction of arsenate to arsenite and determination of total arsenic. Then As(V) is obtained by difference. Nevertheless, there are some papers that claims to have detected it with voltammetry (6),(23).

Table 4. Application examples of modified SPE for Arsenic detection.

Metal ion	Modification	Stripping technique	LOD	Application	Ref.
As(III)	PtNPs	CV	5,68 ppb	Tap water	(19)
As(III)	AuNPs	LSV	0,4 ppb	Canal water	(16)
As(III)	AcH	-	0,8 ppb	Tap water	(24)
As(III)	AuNPs	ASV	0,4 ppb	Certificated water sample	(25)
As(III)	PLA-AuNPs	DPASV	0,09 ppb	Groundwater	(26)
As(III)	Au	ASV	0,03 ppb	water samples from a rice-field	(6)
As(V)	Au	ASV	2,3 ppb	water samples from a rice-field	(6)
As(III)	Ibu-AuNSs	CV	0,018 ppb	Ground, tap and drinking water	(27)
As(III)	AgNPs	DPASV	10 ppb	-	(28)

2.7. Nanoparticles

Nowadays, the development of analytical methods based on nanomaterials is growing attention for numerous applications, including biological research, monitoring of health, clinical diagnostics, pharmaceutical analysis, food safety and environmental monitoring. Due to their small size (normally in the range of 1–100 nm), nanoparticles exhibit unique chemical, physical and electronic properties that are different from those of respective bulk materials, and can be used to construct novel and improved sensing devices (29).

In sensing systems, the most important properties that the nanoparticles offers are :

- the high surface area to volume ratio, which allows enhanced catalytic and sensing response by the rapid movement of analytes through nanomaterials based electrodes or sensors.
- the electrochemical features of the NPs (oxidation or reduction current onto a transducing platform) increases the electrocatalytically active zones on the structure of the sensing device.

Thanks to this two properties combined, the modification of sensors with nanoparticles is really interesting and gaining importance recently. Specifically, SPEs are modified with nanoparticles so that each of the NPs behaves like a nano-electrode; enhancing the analytical signal and thus, improving the detection of the analytes.

2.7.1. Synthesis of nanoparticles

The methods for synthetize nanoparticles can generally involve either "top down" or "bottom up" approaches. In top-down synthesis, nanoparticles are produced by size reduction from a suitable starting material by various physical and chemical treatments. However, top down production methods introduce imperfections in the surface structure of the product and this is a major limitation because the surface chemistry and the other physical properties of nanoparticles are highly dependent on the surface structure.

In bottom up synthesis, the nanoparticles are built from smaller entities, for example by joining atoms, molecules and smaller particles. In bottom up synthesis, the nanostructured building blocks of the nanoparticles are formed first and then assembled to produce the final particle. The bottom up synthesis mostly relies on chemical and biological methods of production. Figure 8 classifies the most used methods for nanoparticle synthesis (30).

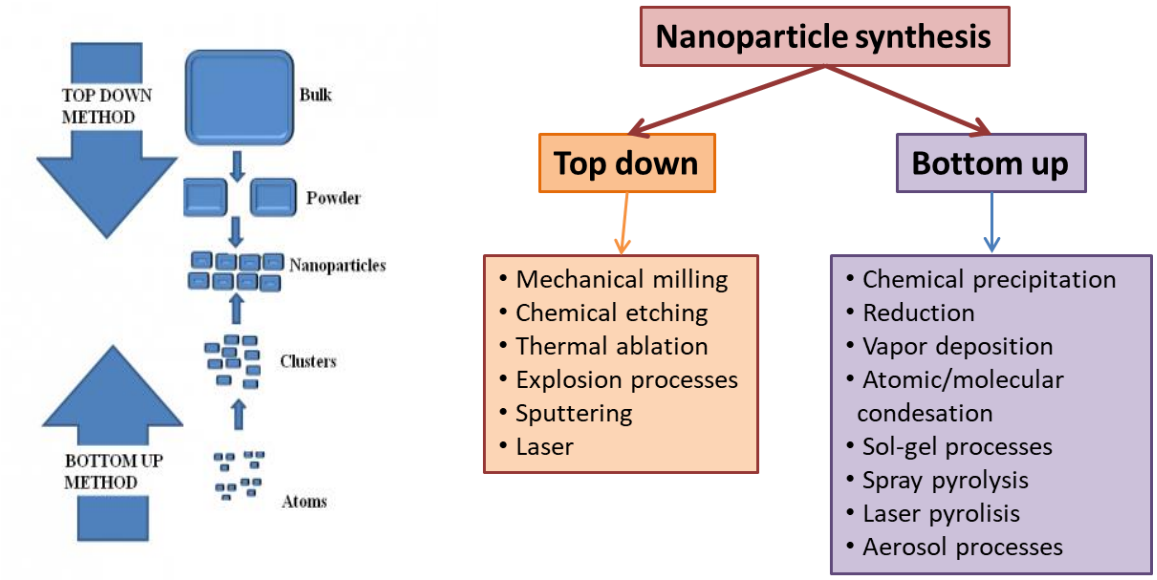


Figure 8. Left: Schematization of top and bottom up synthesis. Right: Approaches of top down and bottom up methods.

2.7.2. Green nanoparticles synthesis

Although the methods described above are successfully used to synthesize nanoparticles, they are expensive and involve the use of hazardous and toxic chemicals. Therefore, there is a growing need to develop environmentally friendly and sustainable methods that could lower the toxicity and the environmental impact of the byproducts. In this way, greener synthesis of nanoparticles provides advancement over other methods as it is simple, cost-effective, and relatively reproducible. In addition, it often results in more stable materials.

Greener synthesis involves using naturally occurring reagents such as vitamins, sugars, plant extracts, biodegradable polymers, and microorganisms as reductants. Among these, plant based materials seem to be the best candidates as they do not need the process of maintaining cell cultures and they are also suitable for large-scale 'biosynthesis' of nanoparticles (31). Plant parts such as leaf, root, latex, seed, and stem are being used for metal nanoparticle synthesis. The key active agent in some of these syntheses are believed to be polyphenols, present for example, in tea, wine and winery waste, red grape pomace (32).

The compounds in the extracts may act as both a reducing agent and a stabilizing agent (capping) in the synthesis of the nanoparticles. First the metal (usually a salt) is reduced to zero-valent metal or metal oxides. Then the particles start growing. The growth of the particle depends on the nucleation and crystal growth rate. Finally the particle is stabilized by capping agents (sugars or phenols) present in the extracts (30).

In particular, silver nanoparticles are the classic and the most preferred target of the above mentioned green methods. This is related to the antibacterial properties of zero-valent silver and easy reduction of Ag(I) salts to form zero-valent silver. Some examples of green reducing agents used for synthetize AgNPs are extracts of hedysarum plant and the sopa-root, extract of *Argemone Mexicana leaf*, leaf extract of *euphorbia hirta L.*, *Euphorbia milii* and *F. vulgare leaves* (32). Recently, the group of *Deptartament d'Enginyeria Química*, *Escola Politècnica Superior* at *Universitat de Girona*, in collaboration with the global project, have synthetized AgNPs from grape stalk, which will be used in this work.

2.7.3. Modification of SPE with nanoparticles

As mentioned before, the employ of nanomaterials in SPEs modification could lead to the construction of new more sensitive and selective electrodes. Among nanomaterials, metallic nanoparticles are the greatest interest due to their unique electronic, optical, electrical and electrochemical properties with potential applications in a wide range of technologies including chemical and biochemical sensors.

Metal nanoparticles dramatically enhance electrochemical sensitivity due to: the roughness of the conductive sensing interface supplying a larger surface area, catalytic properties, and conductivity properties (12). Some metallic oxides and metallic compounds, such as cobalt oxide, gold and platinum nanoparticles have already been reported to be suitable for electrode modifications (33).

Moreover, some authors showed that the stripping voltammetry (ASV) determination of arsenic can be improved by nano-engineering of the electrode surface (16). There are examples of modified SPE with nanoparticles in the previous table (Table 4).

Concretely, modification of SPE surfaces using gold nanoparticles (AuNPs-SPE) has received large attention mainly due to their interesting electrocatalytic properties. The AuNPs can be made by

chemical synthesis, UV light or electron beam irradiation, or electrochemical and can be deposited by adsorption or by electrochemical methods (16). Also, some researchers have prepared PtNPs-SPCE by electrochemically depositing K_2PtCl_6 to detect As(III).

However, such electrodes are costly, display low efficiency and suffer from interference of other metal ions which hinder their commercial applications, specially Au with Cu which is usually found together with arsenic in contaminated waters. Among the various metal nanoparticles, silver nanoparticles (AgNPs) offer themselves as a desirable substrate for the preparation of chemically modified electrodes for electrochemical sensing due to high quantum characteristics of small granule diameter, large specific surface area, and the ability for fast electron transfer (33).

Shahi et al. prepared AgNPs for including in a chitosan modified glassy carbon electrode for detection of As(III) by DPASV. The nanostructure showed high sensitivity with a LOD of 1,20 ppb (34).

Aguirre et al. indicated that silver wire electrodes could be used to detect trace quantities of arsenic in both basic and neutral media with the lowest LOD of 0,09 ppb. Also proved it successfully in tap water (35).

Mukherjee et al. developed AgNPs-SPE that can offer high sensitivity, selectivity and reproducibility for electrochemical detection or As(III) with a LOD of 10 ppb. Moreover, they proved that electrodes modified with ex-situ synthesized AgNPs offered better results as compared to electrodeposited one. The modified electrodes also detects arsenic in presence of interfering metal ions namely Cu, Fe, Zn etc. which are commonly found in drinking water (28).

Along these lines, these electrodes provide an effective option for field monitoring of arsenic in water. Thus, it is proposed for this work to study arsenic detection through DPASV with AgNPs-SPE and more concretely, AgNPs-SPCNFE.

2.7.4. Deposition of nanoparticles on SPE

The nanoparticles can be deposited on the SPE in different ways. The choice of the deposition technique depends on the desired thickness, uniformity, nanomorphology, the substrate (dimension, shape, roughness, wettability) and the material. The most common techniques are drop casting, spin coating, electrodeposition and dip coating.

2.7.4.1. Drop casting

It is the easiest and cheapest technique. It consists of placing drops of the nanoparticle solution on the surface of the SPE with the help of a micropipette. Subsequently, the solvent is evaporated spontaneously or the substrate can be heated to speed up the evaporation process and improve film morphology (Figure 9).

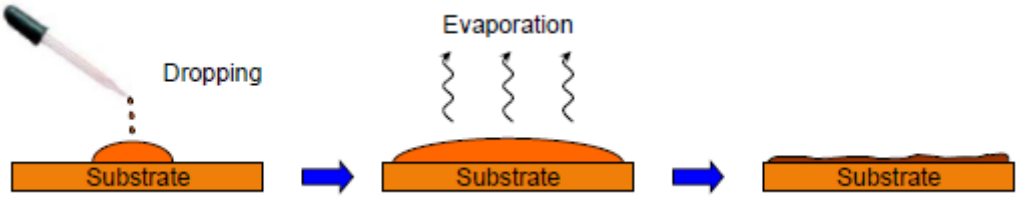


Figure 9. Scheme process of drop casting (36).

The thickness is proportional to the solution concentration. This technique has several advantages as it is a very simple method and no waste of material is made but it has also drawbacks as there are limitations in large area coverage, thickness is hard to control and there is poor uniformity (36).

2.7.4.2. Spin coating

The substrate, which is centered on the chuck of a spin-coater, is covered with a solution containing the dissolved nanoparticles. Upon rotating the sample, centrifugal force spins off the fluid and a uniform film is created (Figure 10). Afterwards, the persistent solvent can be removed by baking the sample on a hotplate at elevated temperatures. The resulting film thickness depends on the molecular weight of the organic material, the concentration of the solution and the spin-speed. The advantages are the good uniformity, reproducibility and good control on thickness. However, there is a waste of material and does not cover a large area (37).

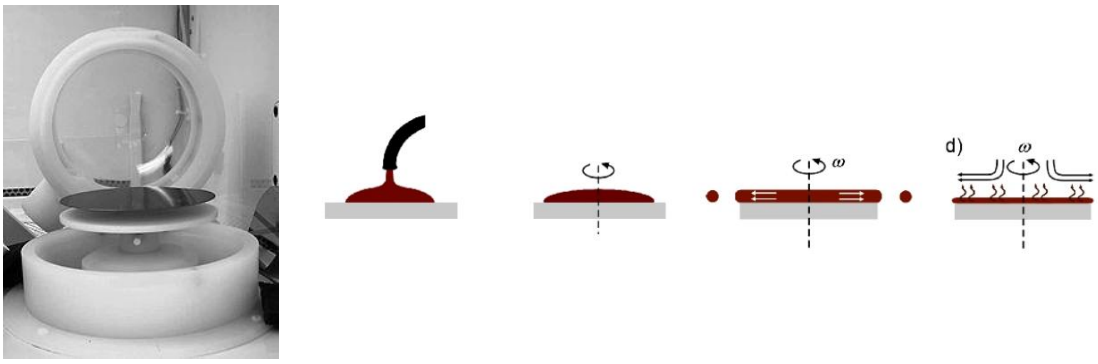


Figure 10. Left: an example of a spin-coater. Right: scheme of spin coating procedure (36).

2.7.4.3. Electrodeposition

Electrodeposition is a long-established way to deposit metal layers on a conducting substrate. Ions in solution are deposited onto the negatively charged cathode, carrying charge at a rate that is measured as a current in the external circuit. The process is relatively cheap and fast and allows complex shapes.

2.7.4.4. Dip coating

The substrate is dipped into the solution or several solutions with different reagents, and then withdrawn at a controlled speed (Figure 11). Thickness is determined by the balance of forces at the liquid-substrate interface. This technique offers quite good uniformity, very thin layers and a large area coverage but there is a waste of material and it is very time consuming (37).

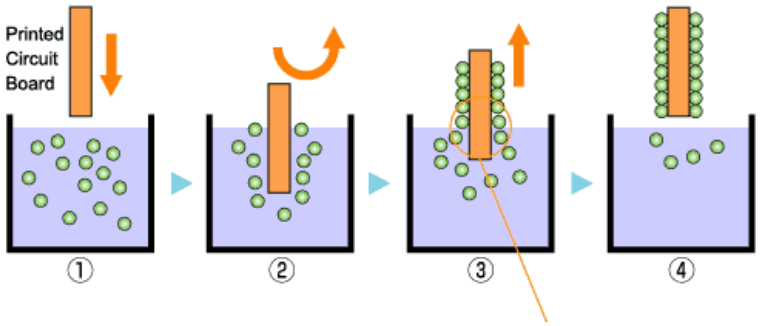


Figure 11. Schematization of dip coating method.

3. OBJECTIVES

This work is a part of a larger project which objective is the green synthesis of metal nanoparticles from acid mine waters and agroalimentary waste extracts. The aim of this current project is to enhance the detection limit of mine water contaminants such as arsenic in very low concentrations, parts per billion (ppb) or µg/l, modifying commercial carbon nanofiber electrodes with two types of silver nanoparticles: synthetized and provided green nanoparticles.

This investigation will include the following objectives:

1. Synthesize silver nanoparticles with different morphology and size in order to attach them into commercial electrodes by drop casting. Also microscopically characterize the nanoparticles.
2. Characterize the modified electrodes.
3. Optimize the experimental conditions for the determination of As(III) and As(V) in aqueous solution through differential pulse anodic stripping voltammetry (DPASV).
4. Evaluate the main analytical parameters of the method by standard addition: regression lines, limits of detection and quantification, linearity range and reproducibility.
5. Perform a screening of the modified electrodes with different nanoparticles types (synthetized and provided green nanoparticles) to determinate which one is the most suitable for the determination of arsenic.
6. Quantify the concentration of a real sample (spiked tap water) with the modified electrodes.

4. EXPERIMENTAL

4.1. Materials/ Chemicals

To carry out the experiments, a number of solutions are required and must be prepared. They are all mentioned below:

- HCl solution 0,01 M (pH = 2).
- 0,1 mol/l acetic acid/sodium acetate buffer solution (pH 4,5)
- Ascorbic acid solution, 1%.
- Daily standard solution of As(III) diluted in the different buffers solutions (dilutions of 1, 10 and 100 ppm).
- Daily standard solution of As(V) diluted in the different buffers solutions (dilutions of 1, 10 and 100 ppm).
- Nitric acid solution 5%.

The reagents used for the preparation of the solutions are:

- Hydrochloric acid 30% from *Suprapur*.
- Standard solution of Arsenic(III) 1000 mg/l for ICP (As_2O_3 in HNO_3, 2%) from *Scharlau.*
- Arsenic(V) Standard for ICP 1001 mg/l from *Fluka Analytical.*
- Ascorbic acid 99,7% from *Scharlau.*
- Sodium acetate 99% from *PanReac AppliChem*.
- Acetic acid 96% from *Merck*.
- Sodium borohydride 96% from *Panreac*.
- Silver nitrate 99% by *Sigma-Aldrich*.
- Sodium styrenesulphonate (PSS) from *Sigma-Aldrich*.
- Aqueous trisodium citrate from *Sigma-Aldrich*.
- Nitric acid 65% from *J. T. Baker*.

The solution of HCl was prepared by adding the appropriate volumes of the 30% HCl solution and make up to the mark with Milli-Q water to the desired volume. The buffer solution was prepared by mixing similar volumes of solutions of acetic acid and sodium acetate trihydrate of concentration 0,1 ml/l to achieve a pH of 4,5.

Daily standard solutions of As(III) and As(V) were prepared by appropriate dilution of stock solutions. Daily standard solutions of Cd (II) and Pb (II) 10^{-3} mol/l were prepared by appropriate dilution of stock solutions, prepared from $Cd(NO_3)_2 \cdot 4H_2O$ and $Pb(NO_3)_2 \cdot 4H_2O$, respectively, and standardized complexometrically.

Ultrapure water (Milli-Q plus 185 system, Millipore) was used in all experiments.

4.2. Experimental setup / instrumentation

DPASV measurements were performed in an *Autolab PGSTAT204* (*EcoChemie*, Utrecht, the Netherlands) and a personal computer with NOVA 2.1 software (*EcoChemie*). The electrochemical cell is supported in the stand that comes with the magnetic stirrer drive Metrohm 801. Figure 12 shows the voltammetric instrumentation.

Figure 12. Examples of the instruments models (all provided by Metrohm) used in the experiments: stirrer, potentiostat and glass cell.

The reference electrode (to which all potentials are referred) and the auxiliary electrode were Ag/AgCl/KNO_3 (0,1 mol/l) and carbon wire, respectively (*Metrohm*, Switzerland) (Figure 13). They were attached to the system by means of flexible cables DRP-CAC from Dropsens.

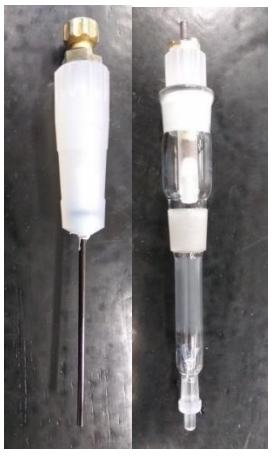


Figure 13. Carbon auxiliary electrode and Ag/AgCl/KNO_3 reference electrode used in the experiments.

The working electrodes were carbon nanofibers modified screen-printed carbon electrode (SPCNFE) devices (Ref, 110CNF) manufactured by *DropSens* (Oviedo, Spain), attached to the system by means of flexible cables *DRP-CAST* also from *Dropsens*. Figure 14 shows an example of SPCNFE.

Figure 14. Carbon Nanofibers modified Screen-Printed Carbon Electrode Ref. 110CNF

Its general dimensions are L33 x W10 x H0,5 mm. The WE has been graphitized with carbon nanofibers to enhance the electrochemical active area. Reference electrode and electric contacts are made of silver and the auxiliary electrode of carbon.

The entire set-up with all the instrumentation needed is shown in Figure 15, and particularly, the cell with the electrodes is shown in Figure 16.

Figure 15. Experimental setup for voltammetric measurements.

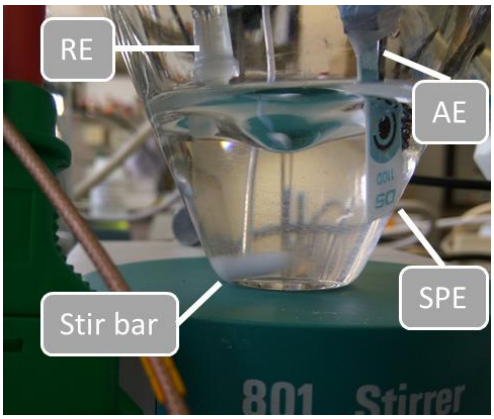


Figure 16. Electrochemical cell with the RE, AE and SPE as WE.

Surface morphology characterization was performed by a scanning electron microscope (SEM) Gemini ZEISS® (Oberkochen, Germany).

For surface characterization of SPCNFE and AgNPs-SPCNFE, *Raman inVia Qontor* microscope (*Renishaw*, United Kingdom) was used.

In the synthesis of AgNPs a syringe pump was used (*Kd Scientific*, model KDS 510).

All measurements were carried out in a glass cell at room temperature (20 °C). Some experiments were under a purified nitrogen atmosphere (Linde N50).

The volume additions for the preparation of the different solutions, as well as for the standard additions in the calibration, are carried out with micropipettes of adequate volume.

4.3. Experimental procedure

4.3.1. Synthesis of Ag nanoparticles

The most used method for synthesis of silver NPs is chemical reduction by organic and inorganic reducing agents. It consists in the reduction of silver ions (Ag^{+}) in aqueous solutions by using different reducing agents such as sodium citrate, ascorbic acid, sodium borohydride ($NaBH_4$) and others that leads to the formation of metallic silver (Ag^{0}), which is followed by agglomeration into oligomeric clusters. These clusters eventually lead to the formation of metallic colloidal silver particles. Besides, protective agents such as polymeric compounds are used to stabilize dispersive NPs during the preparation as they avoid their agglomeration, sedimentation and losing their surface properties (38).

Figure 17 represents the different steps that the synthesis of nanoparticles follows:

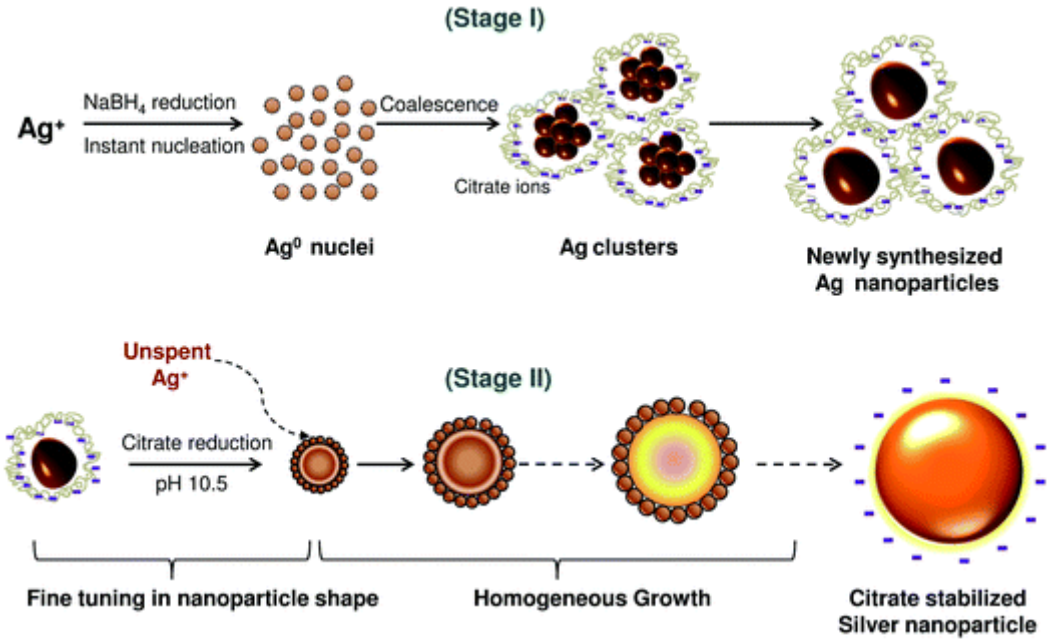


Figure 17. Scheme of the synthesis of nanoparticles (39).

The specific chosen method for silver nanoprism synthesis is by D. Aherne et al. and is a seed-based thermal synthetic procedure that selectively produces (>95%) silver nanoprisms in a rapid and reproducible manner; and under very mild conditions (room temperature and water as solvent) (40).

In this method, $NaBH_4$ is the strong reducing agent that causes the seed nucleation, which leads to the formation of small spherical nanoparticles. Trisodium citrate and PSSS acts as stabilizers. Then, ascorbic acid is the reducing agent (weaker than $NaBH_4$) for the nanoprism formation, with preferential axial growth in crystallization that leads to the formation of the shape defined nanoprisms. For the nanoprism growth, trisodium citrate is the nanoprism stabilizer.

4.3.1.1. Silver seeds production

Ag seeds are produced by combining aqueous trisodium citrate (5 mL, 2,5 mM), aqueous poly (sodium styrenesulphonate) (PSSS; 0,25 mL, 500 mg/L) and aqueous $NaBH_4$ (0,3 mL, 10mM, freshly prepared) followed by addition of aqueous $AgNO_3$ (5 mL, 0,5 mM) at a rate of 2 mL/min while stirring continuously. It results in a clear yellowish solution (Figure 18).

4.3.1.2. Nanoprism Growth

The nanoprisms are produced by combining 5 mL distilled water, aqueous ascorbic acid (75 mL, 10 mM) and different quantities of seed solution (1600 µl and 800 µl), followed by addition of aqueous $AgNO_3$ (3 mL, 0,5 mM) at a rate of 1 mL/min (addition with a syringe pump). After synthesis, aqueous trisodium citrate (0.5 mL, 25mM) is added to stabilize the particles and the sample is

diluted with distilled water as desired. The synthesis is complete after 3 minutes of adding $AgNO_3$, while the color of the solution changes as the surface plasmon resonance red-shifts in response to nanoprism growth.

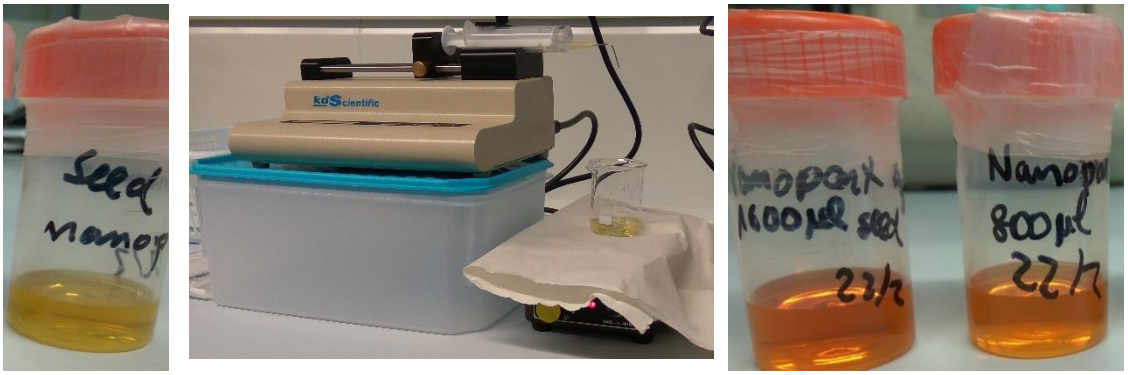


Figure 18. Photos of the three types of AgNPs synthetized (nanoseeds and 1600 and 800 nanoprisms). In the center: process of addition of seed solution quantities with a syringe pump.

4.3.2. Synthesis of "green nanoparticles"

The green nanoparticles were supplied by the group of *Departament d'Enginyeria Química*, *Escola Politècnica Superior* at *Universitat de Girona*, in collaboration with the global project. The biosynthesis of metal nanoparticles is done using grape stalk (waste from winery) and spent coffee (after production of soluble coffee) waste. These agrofood wastes contains a high concentration of reducing agents such as polyphenols and sugars that could be adequate to reduce the metals in solutions to zero-valent and obtain the nanoparticles. The grape stalk and spent coffee extracts are obtained using Milli-Q water as solvent. The extract is then added to a synthetic silver solution to obtain the nanoparticles.

4.3.3. Preparation of modified SPE

The AgNPs-SPCNFE is prepared by drop-casting the AgNPs onto the central WE of the SPE. The amount of the AgNPs solutions that is drop-casted was optimized (41) and found to be 40 µl, resulting in a drop that fully covers the WE. Drop casting simply involves placing drops of the liquid containing the desired material onto the surface of the substrate (SPE) and subsequently allowing the solvent to evaporate (Figure 19). To speed up the evaporation process, the modified SPE is heated in an oven for 30 minutes at 50°C.

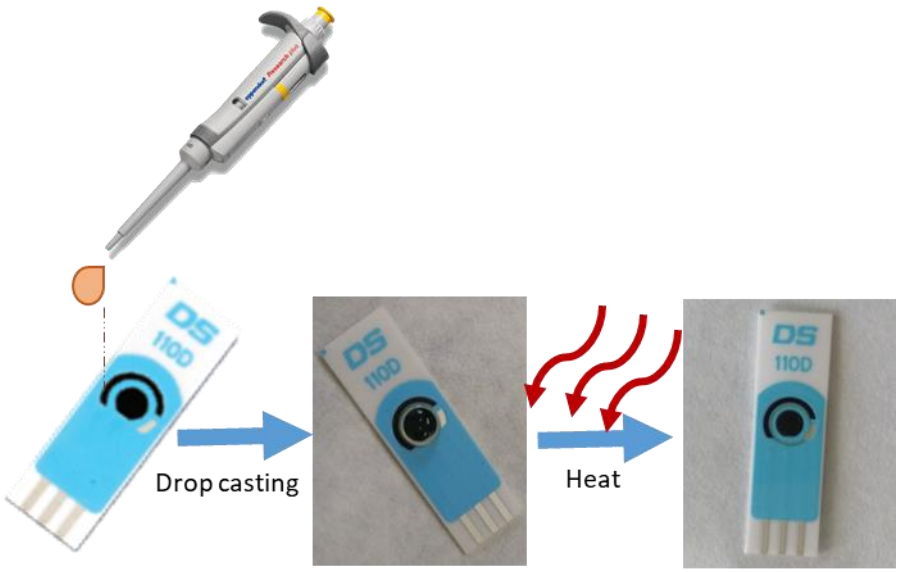


Figure 19. Representation of the steps in the drop casting method.

Also it was checked to drop-casting several layers of nanoparticles to elevate the concentration of silver in the electrode. The method consisted in repeat 3 times the previous method but 20 µl each layer, with a total of 60 µl (3 layers).

4.3.4. Mounting SPE

The procedure for assembly the SPE as working electrode consists in the following steps:

First, an electrode in a good condition was selected, i.e., having a carbon surface well-polished and without visible scratches.

Once selected the electrode, it was properly connected in the cable connector also provided by DropSens. This cable links the electrode and the Autolab.

The WE connected to the cable will be covered with *parafilm*, as a precaution, to avoid any contact with the solution in the cell.

4.3.5. Software for voltammetric measurements

4.3.5.1. Setting the template

The potentiostat (Autolab PGSTAT204) was controlled by computer with NOVA software. Before any experiment, a template for the procedure was made for all the experiments. The template contains the steps that a DPASV must follow and it is configured with icons which represents the commands. When click-in the icons, the command parameters can be changed, which some of

them were constant but there were also variable parameters. The variable are the ones that were changed for certain experiments, for example, for the determination of the optimal E_d, the E_d value was changed.

The home page of NOVA is in Figure 20. The left column has the options for creating new procedures or for loading previous ones and data. The lower part shows the connected accessories that the software can control. For creating the template, click-in the “new procedure” on the left column.

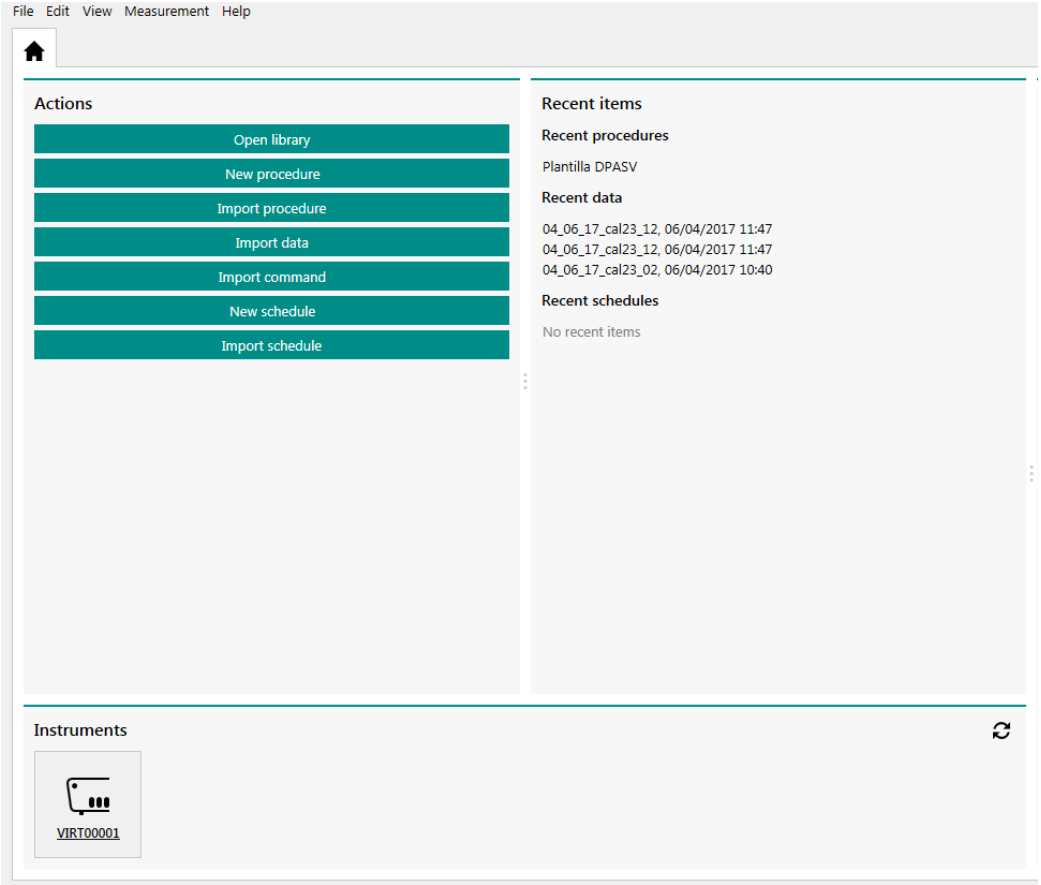


Figure 20. Home page of NOVA software 2.1 version.

The procedure steps are set by dragging the command icons in the middle part of the template. The finished template for the DPASV has the following aspect/configuration (Figure 21). Note that in the figure, there are some commands marked with a red exclamation meaning that the command cannot take place as the screen-shoot was taken when the instrumentation was not connected.

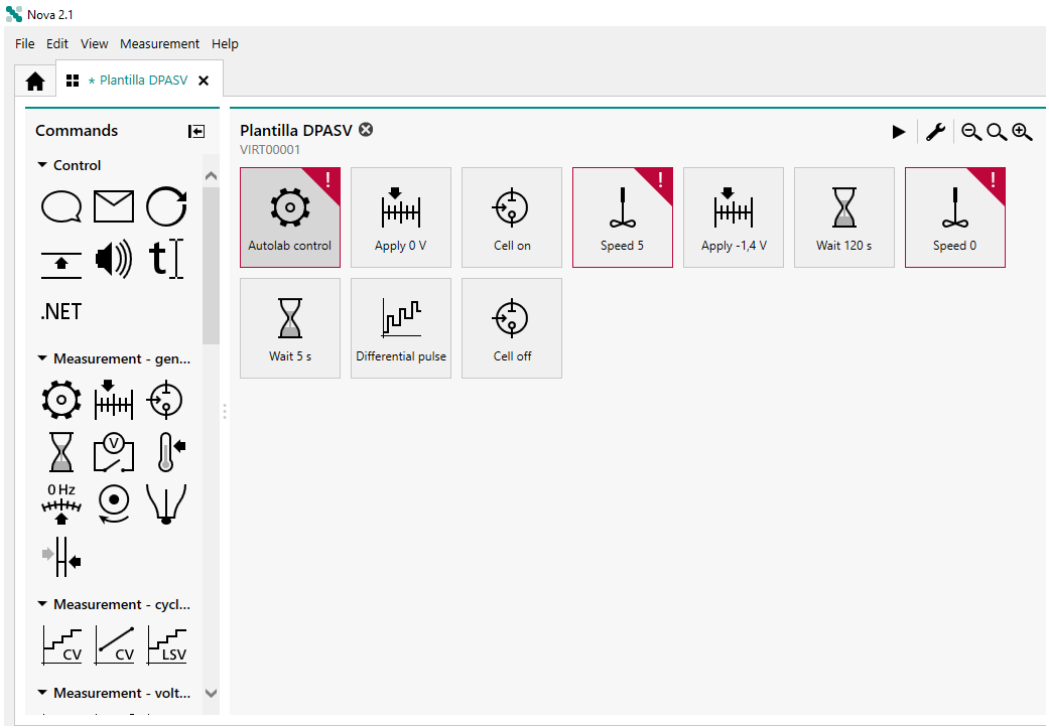


Figure 21. DPASV template configuration.

The first three icons command to power the cell. Then the speed of the stirrer is set with a number which has been found in previous experiments that the optimal is 5. The deposition step is followed and set with the command "Apply" where E_d can be changed (Figure 22).

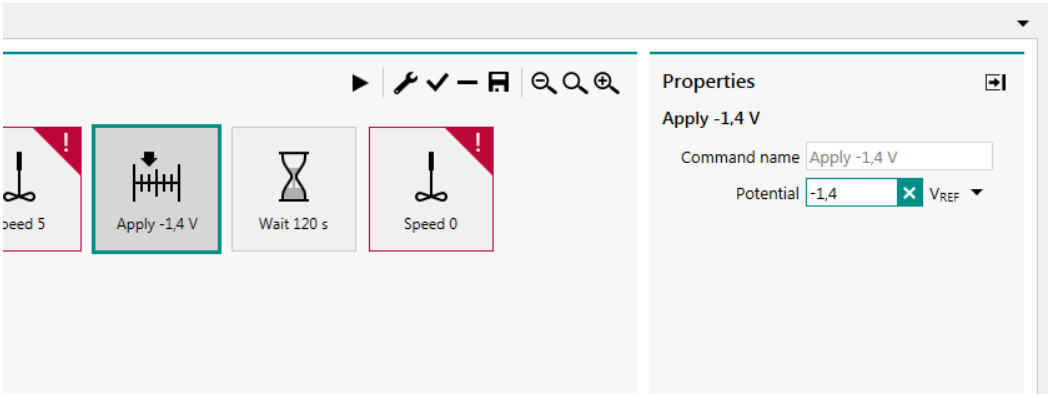


Figure 22. Detail of the deposition step of the DPASV template.

The time of the deposition step is set with the "wait" command, which will be constant and 120 s because all the determination has to be fast and practical. In any case, it was found that 2 minutes is enough time to get good signal.

Once the deposition time is over, the stirring must be stop ("speed 0") as in the stripping step there should be no convection. Because of it, the solution is stabilized with 5 s of repose ("wait 5 s").

Then the stripping step is set with the "differential pulse" command. When click-in on it, the different parameters appears on the right column and must be defined (Figure 23). In this command, only the E_i and the E_f were changed through the experiments.

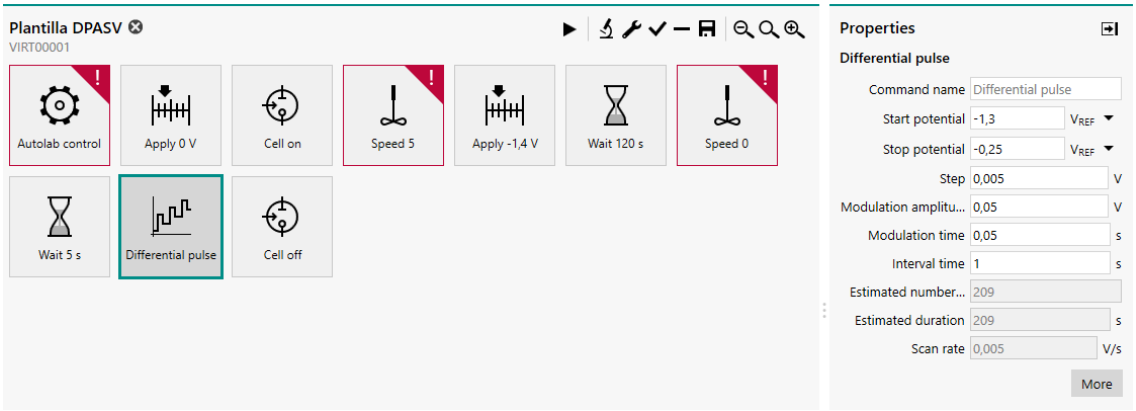


Figure 23. Properties of the differential pulse step.

Finally the cell is powered off with the "cell off".

4.3.5.2. Procedure with NOVA for the voltammetric measurements

Once the template is configured, for any experiment of voltammetry with the DPASV technique the following steps were followed:

1. Open the template.
2. Define the E_d on the "apply" command.
3. Set the E_i ("start potential") and the E_f ("stop potential) on the properties in the differential pulse command.
4. Start the DPASV by click-in the play button on the top right.
5. While running the differential pulse, a plot is generated, showing the measured current vs the potential applied (Figure 24).

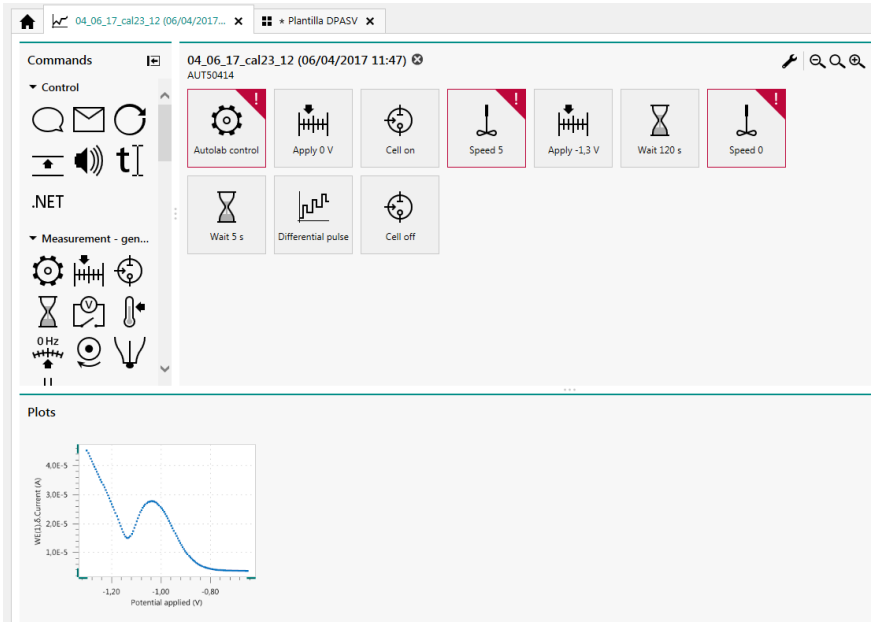


Figure 24. Example of the data tab that it is generated when running the template**.**

6. Once the DPASV is finished, data of the DPASV experiment can be exported by click-in on the plot 2 times (Figure 25).

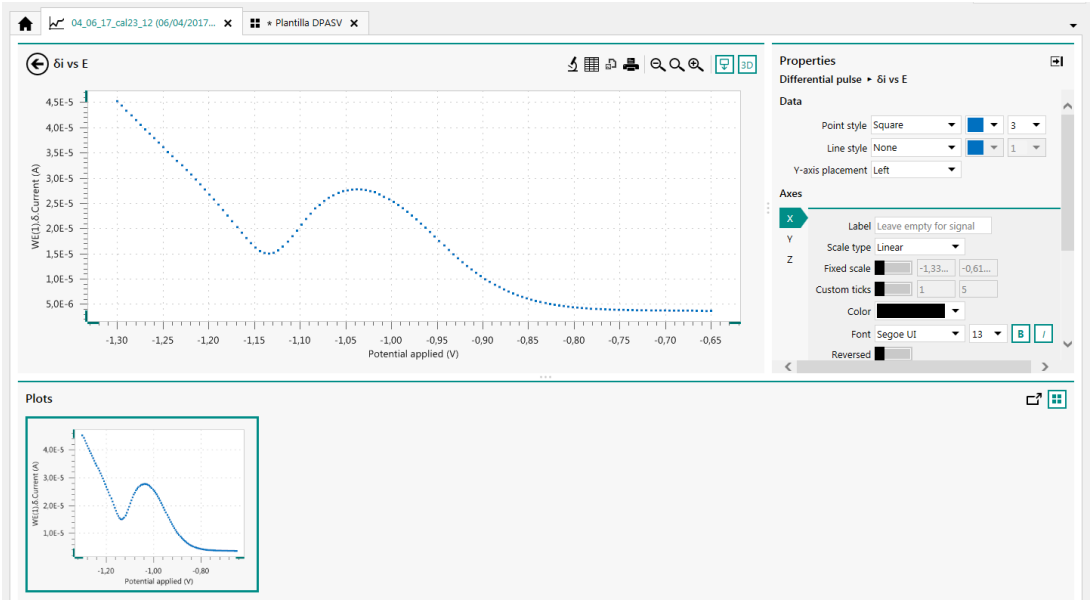


Figure 25. Plot of current measured vs potential applied that the NOVA software generates for a DPASV measurement with the template.

7. The first icon is an analysis command which contains the peak search, which can determine the peak position, height and area values. The peak analysis can be manual or automatic. For all the experiments, the peak analysis mode was manual as the automatic mode detects wrongly that the baseline is lineal. The chosen baseline was polynomial and it is generated

by manually clicking on three points on the plot (initial point, highest height and final point of the baseline) (Figure 26).

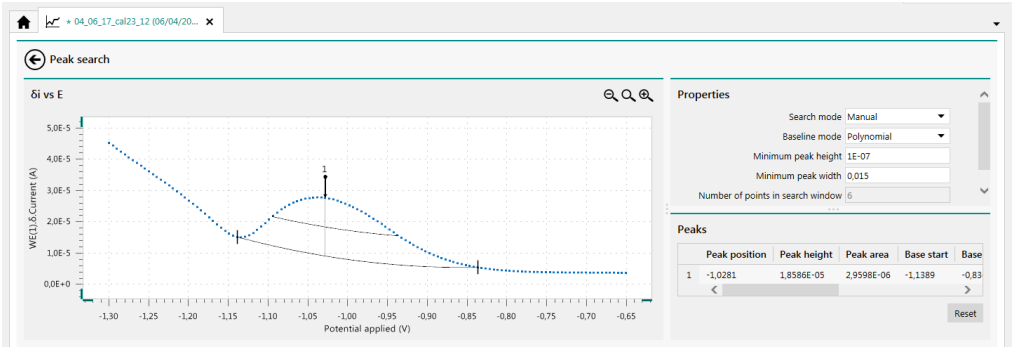
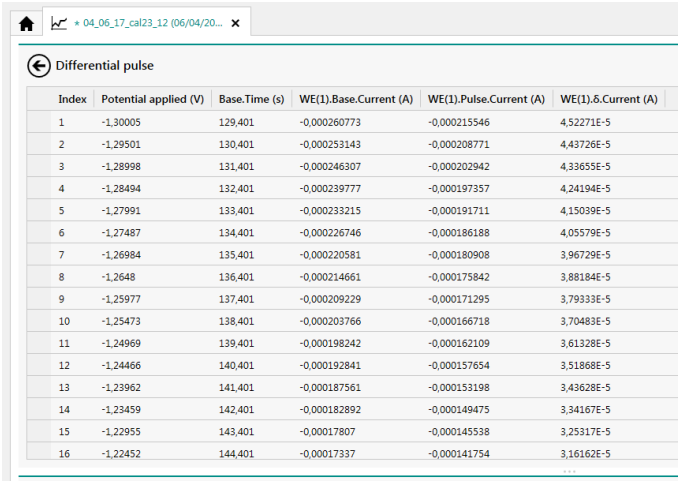


Figure 26. Peak search option on the data tab generated.

8. The second icon generates a table with the WE and the V (Figure 27).



Differential pulse

Index	Potential applied (V)	Base.Time (s)	WE(1).Base.Current (A)	WE(1).Pulse.Current (A)	WE(1).δ.Current (A)
1	-1,30005	129,401	-0,000260773	-0,000215546	4,52271E-5
2	-1,29501	130,401	-0,000253143	-0,000208771	4,43726E-5
3	-1,28998	131,401	-0,000246307	-0,000202942	4,33655E-5
4	-1,28494	132,401	-0,000239777	-0,000197357	4,24194E-5
5	-1,27991	133,401	-0,000233215	-0,000191711	4,15039E-5
6	-1,27487	134,401	-0,000226746	-0,000186188	4,05579E-5
7	-1,26984	135,401	-0,000220581	-0,000180908	3,96729E-5
8	-1,2648	136,401	-0,000214661	-0,000175842	3,88184E-5
9	-1,25977	137,401	-0,000209229	-0,000171295	3,79333E-5
10	-1,25473	138,401	-0,000203766	-0,000166718	3,70483E-5
11	-1,24969	139,401	-0,000198242	-0,000162109	3,61328E-5
12	-1,24466	140,401	-0,000192841	-0,000157654	3,51868E-5
13	-1,23962	141,401	-0,000187561	-0,000153198	3,43628E-5
14	-1,23459	142,401	-0,000182892	-0,000149475	3,34167E-5
15	-1,22955	143,401	-0,00017807	-0,000145538	3,25317E-5
16	-1,22452	144,401	-0,00017337	-0,000141754	3,16162E-5

Figure 27. Example of a table of the current measured vs the potential applied that the NOVA generates for a DPASV experiment.

4.3.6. Voltammetric determination of As (III)

The procedure followed for all the experiments of arsenic quantification in an aqueous solution is detailed below but first, all the steps previously explained must be done (synthesis of the solutions and the nanoparticles, preparation and modification of the SPE with AgNPs, mount the SPE, create the template and configure it).

Then, a cell previously cleaned with a 5% solution of nitric acid is filled with a volume of 35 ml of 0,01 M hydrochloric acid as it was found in literature to be a good medium for the determination of arsenic (42). Also it was found that As(III) is sensitive to oxidation, thus 175 µl of ascorbic acid were added to stabilize the As(III) and the sample is only added after the hydrochloric acid has been degassed.

The required amount of arsenic is then added to give a desired concentration. The mounted SPE is introduced into the cell, as well as a stir bar for magnetic stirring and the external electrodes.

Then the procedure template is loaded in the NOVA software. The variable parameters (E_d, Ei and E_f) must be chosen and defined. Once ready, the procedure can be started.

As mentioned before, DPASV has to main steps:

- Deposition/pre-concentration. In the first step, the arsenic ions in the HCl solution are concentrated and deposited onto the working electrode of the SPE by electrolysis at E_d. The concentration of the analyte on or in the electrode is therefore much higher than the concentration in the solution. Stirring was necessary in this step and the deposition time t_d was 120 s.
  The reduction that takes places is the following (Eq. 5):

$$\boldsymbol{As^{3+} + 3\ e^{-} \rightarrow As^{0}} \qquad \textbf{(Eq. 5)}$$

- Stripping. Before starting the stripping step, the stirring must be stopped but E_i remains constant. This helps the analyte to homogeneously distribute on the electrode. After deposition, a rest period (t_r) of 5 s is followed and then the potential was scanned from E_i to E_f (final deposition potential) using pulse times of 50 ms, step potentials of 5 mV and pulse amplitudes of 50 mV.
  During stripping step, the deposited analyte is determined by differential pulse by stripping itself, through a potential sweep in the opposite direction to the initial (Eq. 6):

$$As^{0} \rightarrow As^{3+} + 3\ e^{-} \qquad \textbf{(Eq. 6)}$$

Once the DPSV finishes, the obtained data is exported to Excel. The used SPE can be discarded, the content of the cell must be disposed of in the acid solution with heavy metals container and finally the cell must be cleaned after each experiment.

4.3.7. Glassware and cleaning protocol

Since the work is carried out with extremely low concentrations of metal, it is important to clean all the material properly as a minimum contamination can lead to significant errors.

The cell must be cleaned each time, first rinsing with Milli-Q water and then the cell must be filled with solution of nitric acid 5% for a night. After that, just before using the cell, it has to be rinsed again with Milli-Q water and dried with special wipes.

Furthermore, the electrolyte of the external electrodes must be renovated each time.

4.3.8. Calibration curve and standard addition

Calibration curves were carried out to prove that the AgNPs-SPCNFEs can detect arsenic and the signal obtained is lineal with the concentration. The calibration curves were constructed by measuring the heights or areas of the peaks (y axis) obtained in the voltamperograms (current signal vs applied potential) for each known analyte concentration (x axis). Figure 28 shows a representative voltammogram, which is used for the construction of the calibration curve also in the figure.

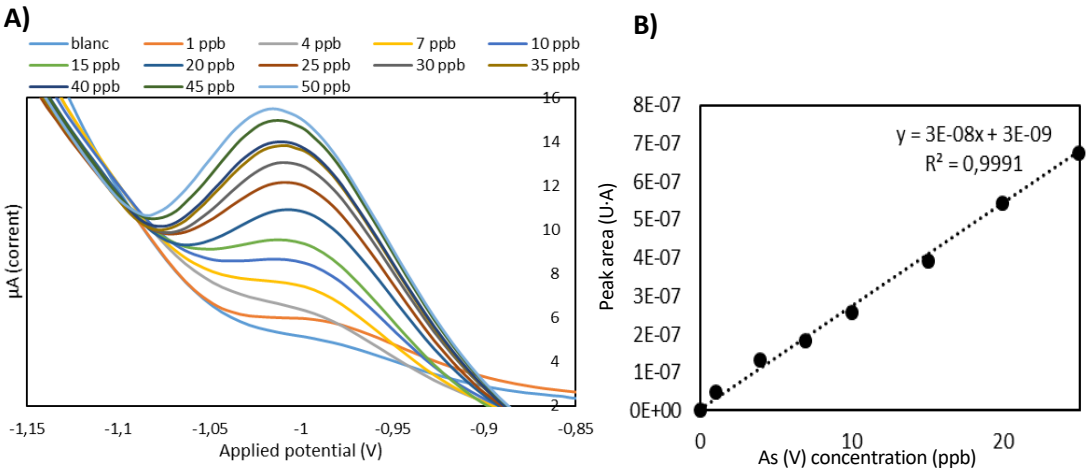


Figure 28. A) Example of voltamperogramm. B) Example of calibration curve.

The chosen method for determining the concentration of arsenic in an unknown sample or real sample is by standard addition calibration method, which consists in adding standard solutions of arsenic of known concentration in the real sample. In the standard addition plot, the point at zero concentration added As is the reading of the unknown, the other points are the readings after adding increasing amounts ('spikes') of standard solution. The absolute value of the x-intercept is the concentration of As in the unknown (the real sample).

4.3.9. Detection limits

Detection limits are terms used to describe the smallest concentration of an analyte that can be reliably measured by an analytical procedure and are very useful in analytical chemistry. The detection limits calculated in this work are the following:

- Limit of detection (LOD) is the lowest quantity of a substance that can be distinguished from the absence of that substance (a blank value) within a stated confidence limit.
- The limit of quantification (LOQ) of an individual analytical procedure is the lowest amount of analyte in a sample which can be quantitatively determined with suitable precision and accuracy

Detection limits were calculated using Millers & Miller's methodology which is expressed as the concentration whose signal in the instrument is equal to the mean of the target signals plus the triple of the standard deviation of these (Eq. 7) and (Eq. 8):

$$\boldsymbol{LOD = \frac{3,3 \cdot \sigma}{S}} \quad \textbf{(Eq. 7)}$$

$$\boldsymbol{LOQ = \frac{10 \cdot \sigma}{S}} \quad \textbf{(Eq. 8)}$$

Where:

- σ : standard deviation of the response
- S: slope of the calibration line

5. RESULTS

5.1. Characterization of Ag nanoparticles

The AgNPs were obtained with a seed-based method, which first involves the crystallization of nanoseeds due to the reducing agent $NaBH_4$. Then, this nanoseeds can be used for another nucleation promoted by ascorbic acid to obtain shaped nanoprisms of larger size (41).

Figure 29 shows images obtained by SEM of the synthesized samples of Ag NPs, thus corroborating the effective formation of the same (as it can be identified as the white spherical dots). Also in the figure, it can be seen indicated the diameter of some of the nanoparticles calculated by software, which are between 8 and 13 nm for the nanoseeds, ±20 nm for the Ag nanoprisms 800 and ±22 nm for the Ag nanoprisms 1600.

For the morphology study of the nanoparticles, TEM offers better images as the shapes can be recognized. Figure 30 which is from the group of electroanalysis of Universitat de Barcelona (41) provides the characterization of spherical Ag nanoseeds and shaped defined Ag nanoprisms. Also it incorporates size distribution histograms, with similar diameters found with SEM images. Nanoseeds have spherical shape, whereas nanoprisms have triangular shape with polycrystalline structure.

Furthermore, SEM images of the “green” nanoparticles (environmentally friendly NPs) were provided by Cristina Arenas which TFG was about the synthesis of them (Figure 31). It can be appreciated that with the similar magnifications there is less density of NPs, which can affect to the determination of the metal. Also, there is more size distribution and the morphology is quite different.

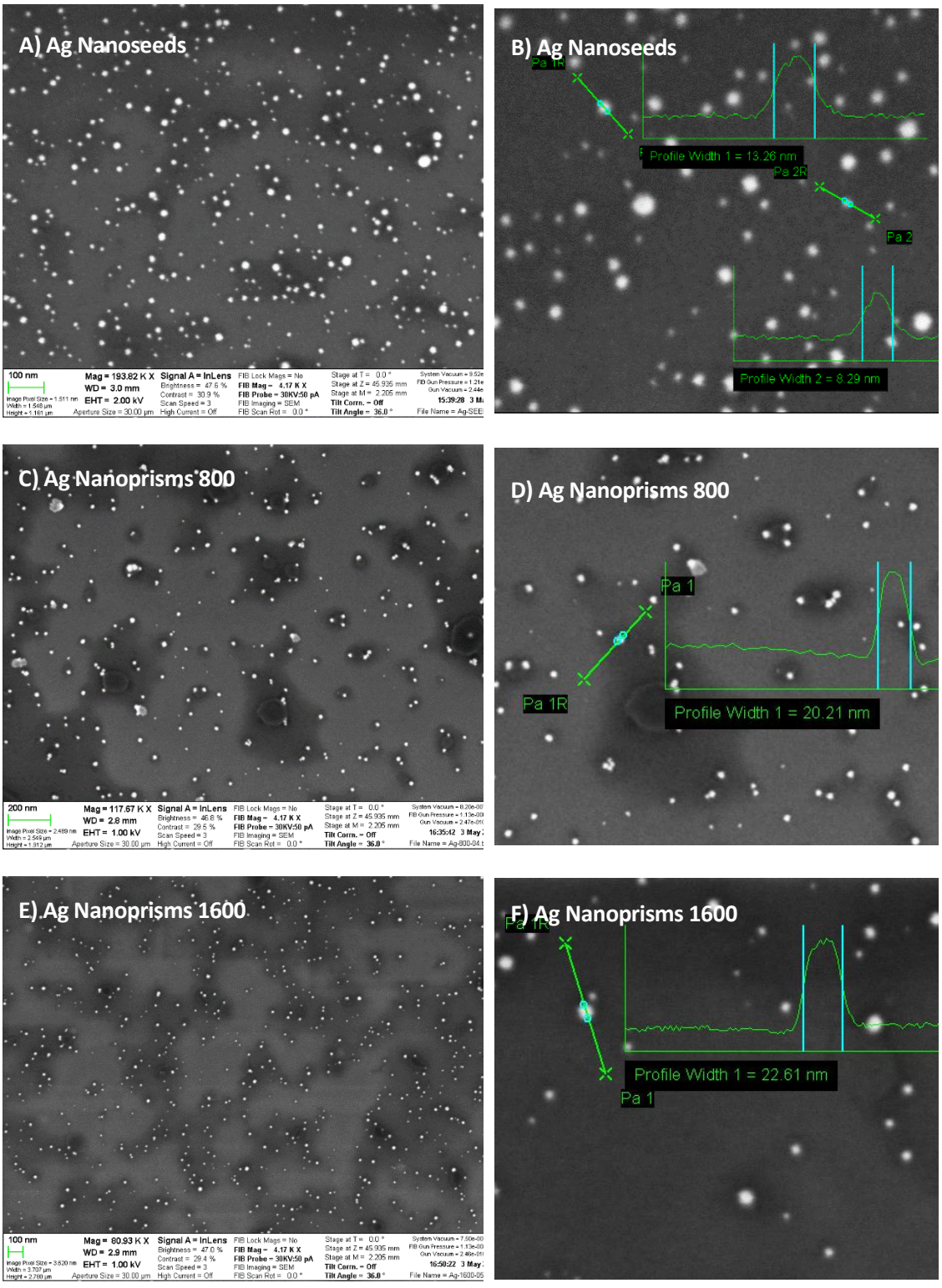


Figure 29. Images obtained by SEM of the synthesized samples of AgNPs (nanoseeds (A,B), nanoprisms 800 (C,D) and nanoprisms 1600 (E,F)) with a screenshot of the calculated diameters.

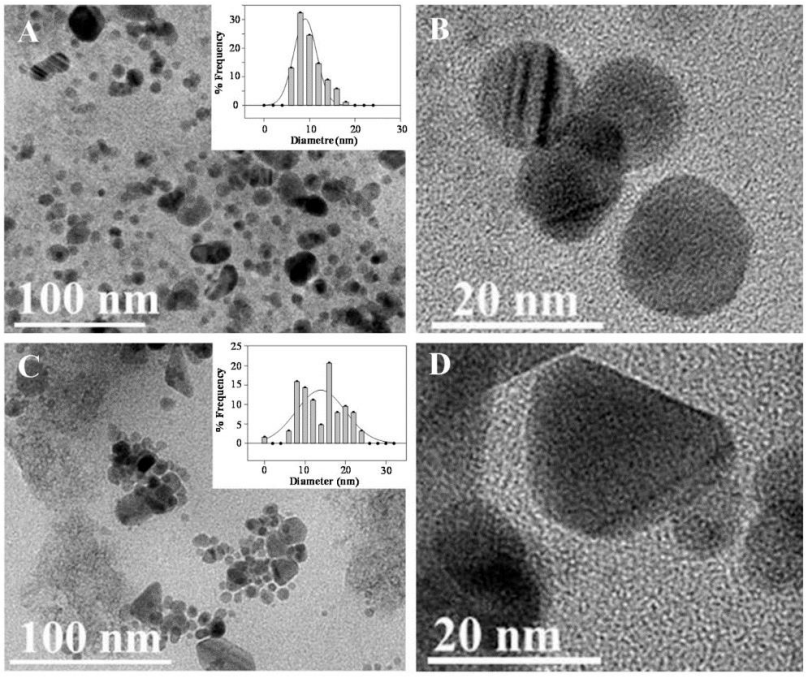


Figure 30. TEM micrographs of 9.1 ± 0.4 nm spherical Ag-nanoseeds (A,B) and triangular shaped 14.0 ± 0.9 nm Ag-nanoprisms (C,D) with the corresponding size distribution histograms (insets in A and C) (41).

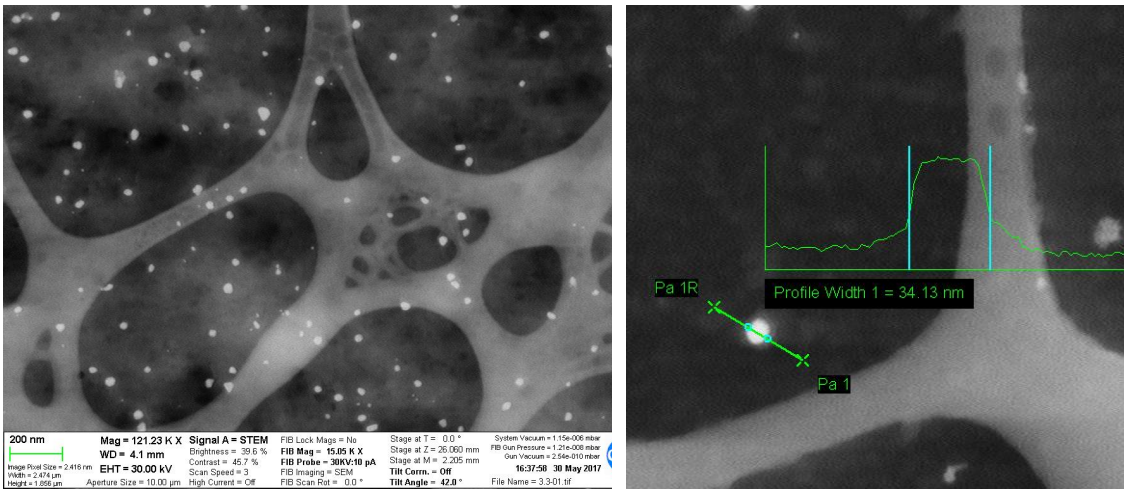


Figure 31. SEM images of the nanoparticles synthetized by green route courtesy of Cristina Arenas.

5.2. Characterization of AgNPs-SPCNFE

Figure 32 shows different images of the WE of a bare SPCNFE, obtained with *Raman inVia Qontor* microscope (*Renishaw*, United Kingdom). It can be appreciated that the printing is not regular and homogenize. In addition, there are holes without carbon ink that can affect the reproducibility of the experiments.

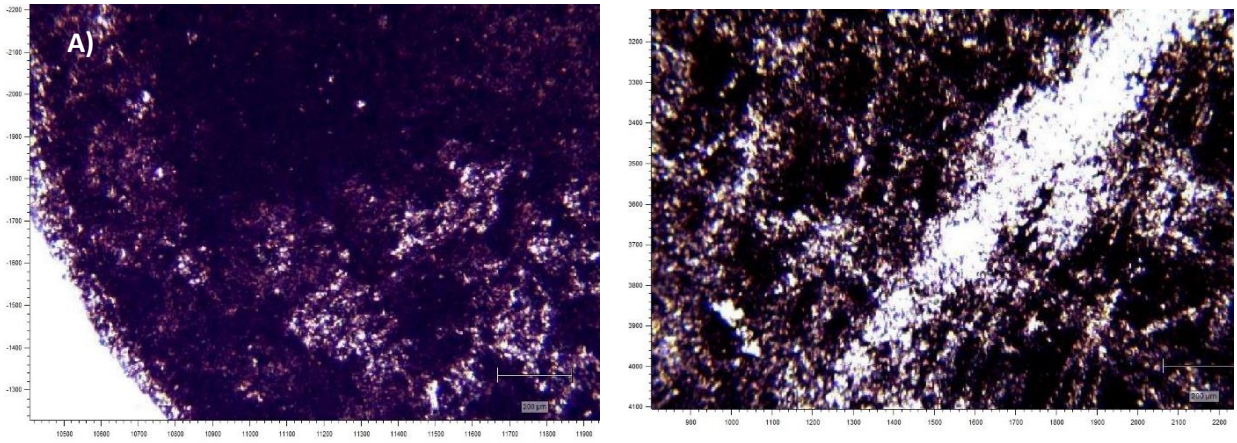


Figure 32. Optical microscopic images of an SPCNFE obtained with *Raman* microscope.

Furthermore, the bare SPCNFE have been examined with SEM. Figure 33 shows the electrode at 3K X (Figure 33A) and 100K X (Figure 33B). In A, it can be noticed that the structure it is not regular and in B, the nanofibers are really visible. This structure will help retain and adsorb the AgNPs that will be added for better analysis of the metal.

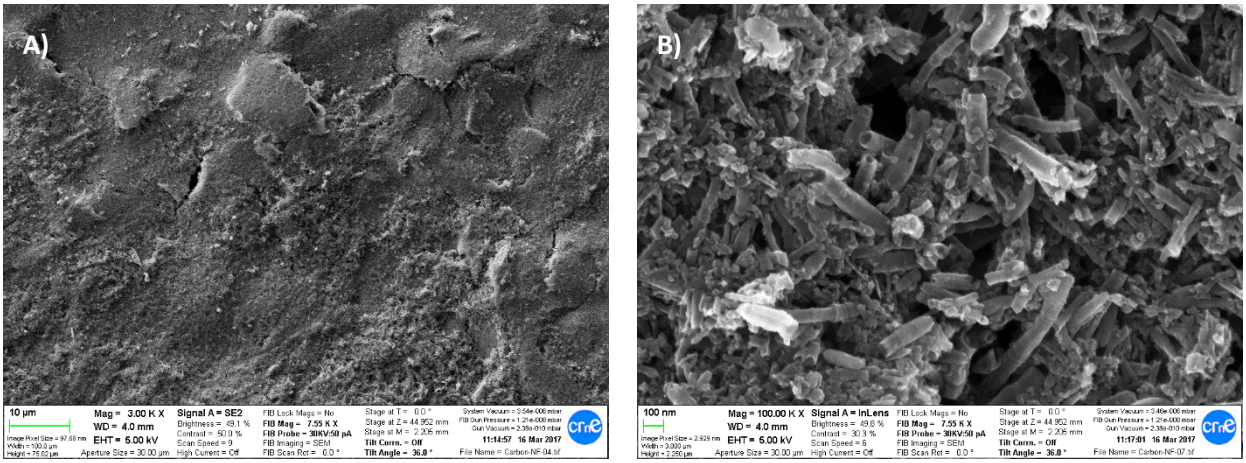


Figure 33. SEM images of non-modified SPCNFE at 3K X (A) and 100K X (B).

Due to the irregular structure, it should be noticed that although the screened electrodes already have auxiliary reference electrodes, it is preferred to work with the aforementioned external auxiliary and reference electrodes to ensure the goodness of the measurements.

SEM images at 15K X of the SPCNFE surface modified with AgNPs (Figure 34B) show, in comparison to the bare SPCNFE (Figure 34A), the presence of NPs all over the electrode surface (as white dots). This can explain the capability to improve the electrochemical performance in comparison with the non–modified SPCNFE. Figure 34C and D shows the WE at 100K X. The two images focus the same

spot of the SPE, but the nanoparticles are more visible at the unfocused one. In Annex, there are some of these images with better resolution.

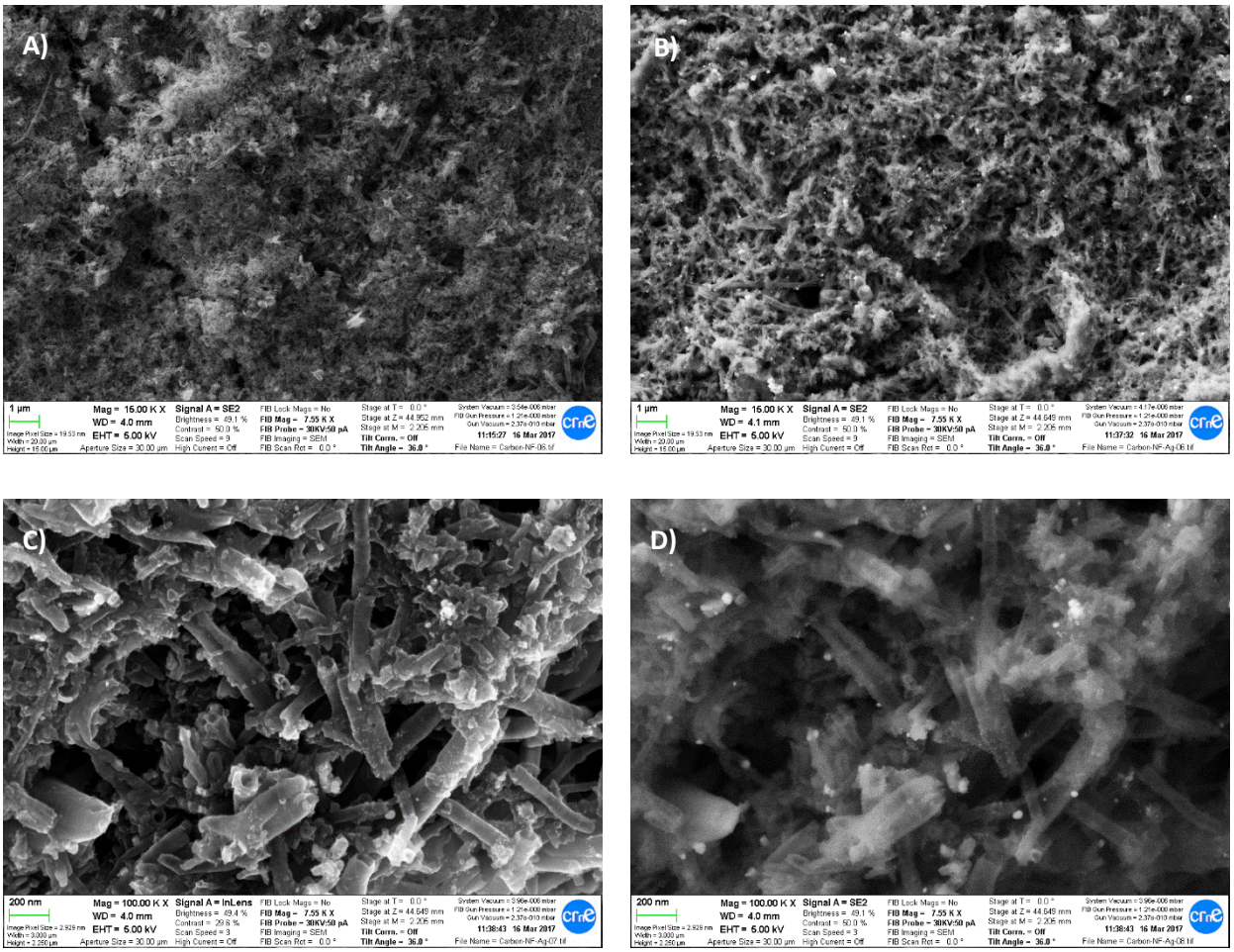


Figure 34. SEM images: A) non-modified SPCNFE at 15K X . B) AgNPs- SPCNFE at 15K X. C) and D) AgNPs-SPCNFE at 100K X.

5.3. Determination of As(III)

Initially, the optimal electrochemical parameters were determined for As(III) determination. In this case, the chosen nanoparticles were Ag-nanoprisms 800. An initial calibration test was performed with an E_d of -0,35 V (28) and arsenic concentration range up to 150 ppb in 12 intervals. It was found that a peak grew with arsenic concentration around -0,1 V (Figure 35).

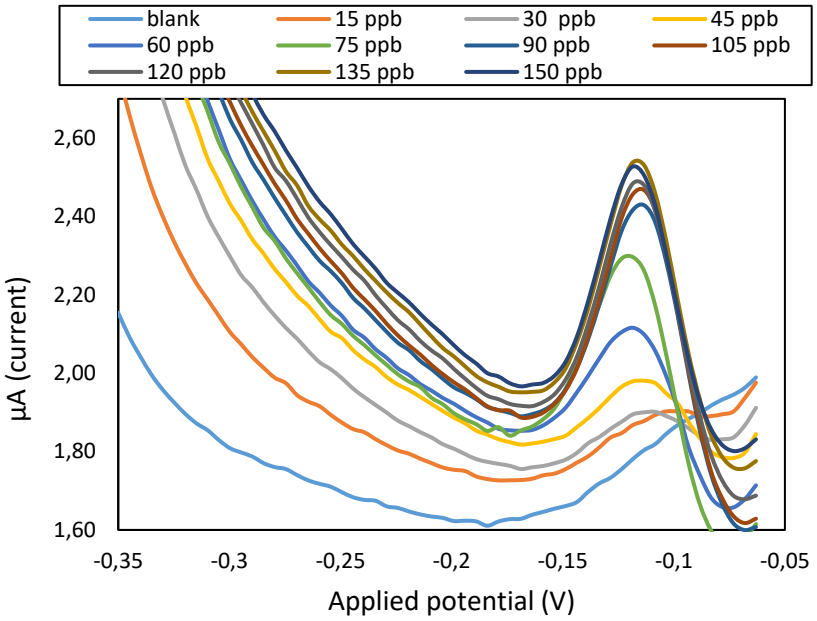


Figure 35. Stripping voltammetric measurements of As(III) with range concentrations up to 150 ppb in a solution of HCl (pH = 2) using an E_d of −0,35 V during a t_d of 120s on Ag-nanoprisms 800-SPCNFE.

Then, for the optimization of E_d, stripping measurements of a solution containing 150 ppb of As(III) in HCl pH = 2 were carried out at several deposition potentials (E_d) ranging from −0,3 to −0,55 V and using a deposition time (t_d) of 120s. The optimal E_d was found to be -0,5 V since although the peak height is very similar to that found for -0,55 V, the area is higher for -0,5 V. (Figure 36).

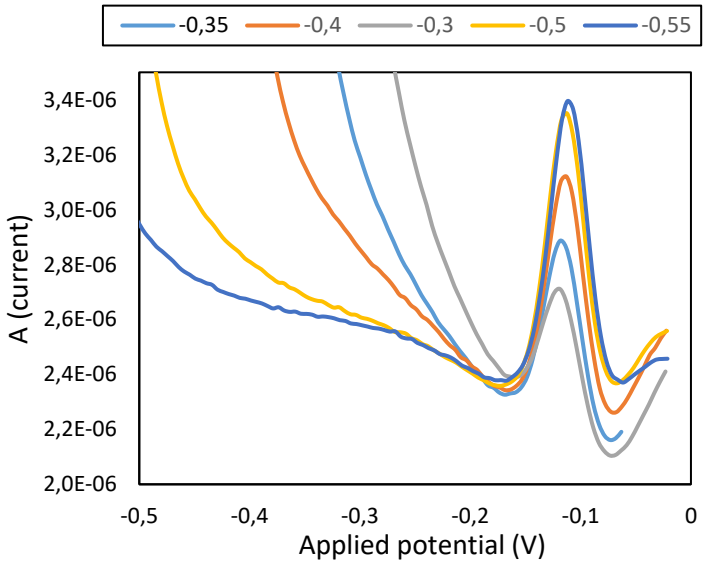


Figure 36. Stripping voltammetric measurements of As(III) at 150 ppb for the optimization of E_d with Ag-nanoprisms 800-SPCNFE.

The problem is that similar repetitions were not achieved as the peaks were moved to the right, or be split in two (Figure 37), a signal that As(III) is being oxidized. For this reason, it was decided to continue with As(V) to find its optimum conditions.

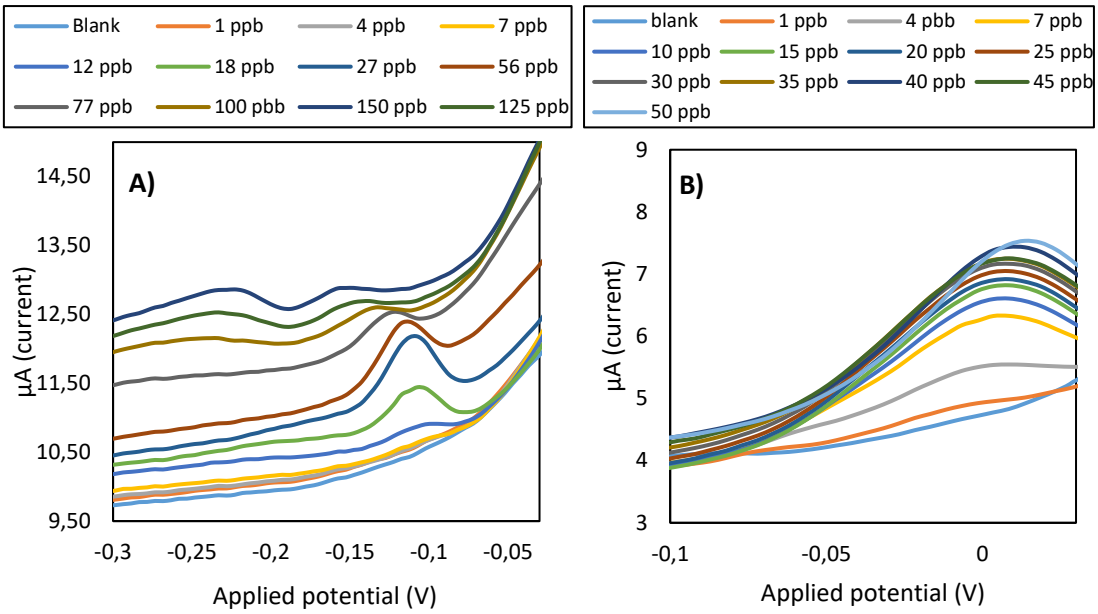


Figure 37. A) Stripping voltammetric measurements of As(III) with range concentrations up to 150 ppb in a solution of HCl (pH = 2) using an E_d of −0,35 V during a t_d of 120s on Ag-nanoprisms 800-SPCNFE. B) repetition.

5.4. Optimization of the voltammetric parameters for As (V)

The determination of the optimal conditions for stripping experiments with As(V) were performed just as for As(III). First, the peak is searched increasing the concentration of arsenic up to 200 ppb and with a constant deposition potential found in the bibliography (E_d = -1,2 V). Figure 38 shows that the peak is located at -1 V approximately, and also proves that the peak grows with the concentration.

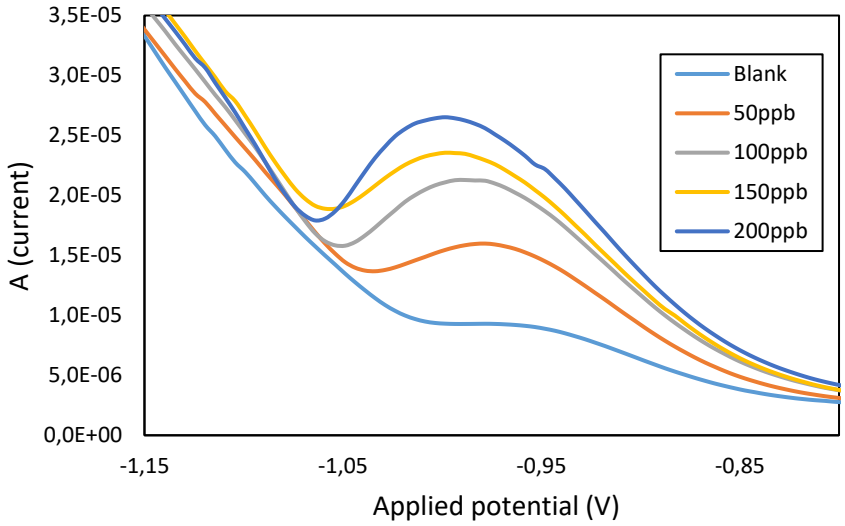


Figure 38. Stripping voltammetric measurements of As(V) with a range of concentrations up to 200 ppb in a solution of HCl (pH = 2) using an E_d of −1,2 V during a t_d of 120s on Ag-nanoprisms 800-SPCNFE.

Then, different deposition potentials are applied to find the optimal E_d at a constant concentration of arsenic of 200 ppb (Figure 39), which results to be -1,3 V for the highest absolute peak height.

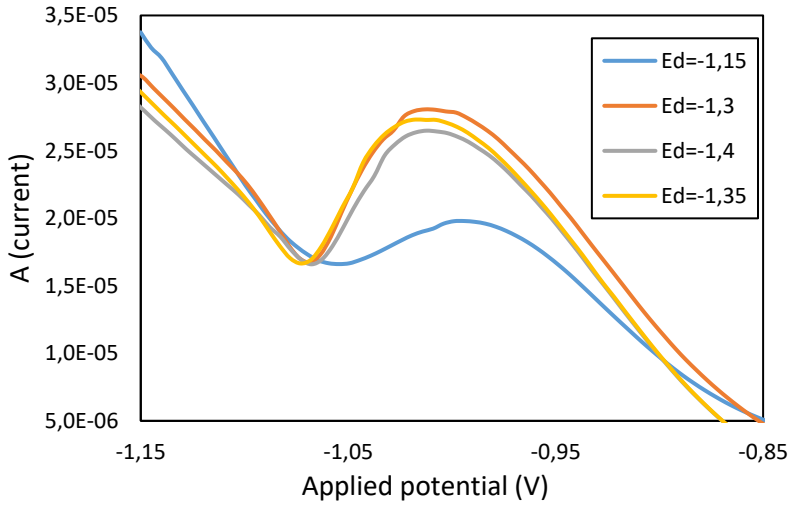


Figure 39. Stripping voltammetric measurements of As (V) at 200 ppb for the optimization of E_d with Ag-nanoprisms 800-SPCNFE.

The acetic acetate buffer was also tested, which is the most common in voltammetry for metal ions. However, no peak was found, which concludes that the presence of chloride ions are necessary for oxidation / reduction of the metals.

5.5. Non-modified SPCNFE

In order to prove the enhancement of the electrochemical response due to the AgNPs attached to the SPE, firstly, measurements of As(III) and As(V) were carried out on a non-modified SPCNFE. The results are showed in Figure 40. In the case of As(V), the results were inconsistent as the peak was decreasing when increasing the concentration, maybe due to the own oxidation of the WE as it has no protective coating. In the case of As(III), the peaks were small and formless despite of the high concentration of arsenic that was added.

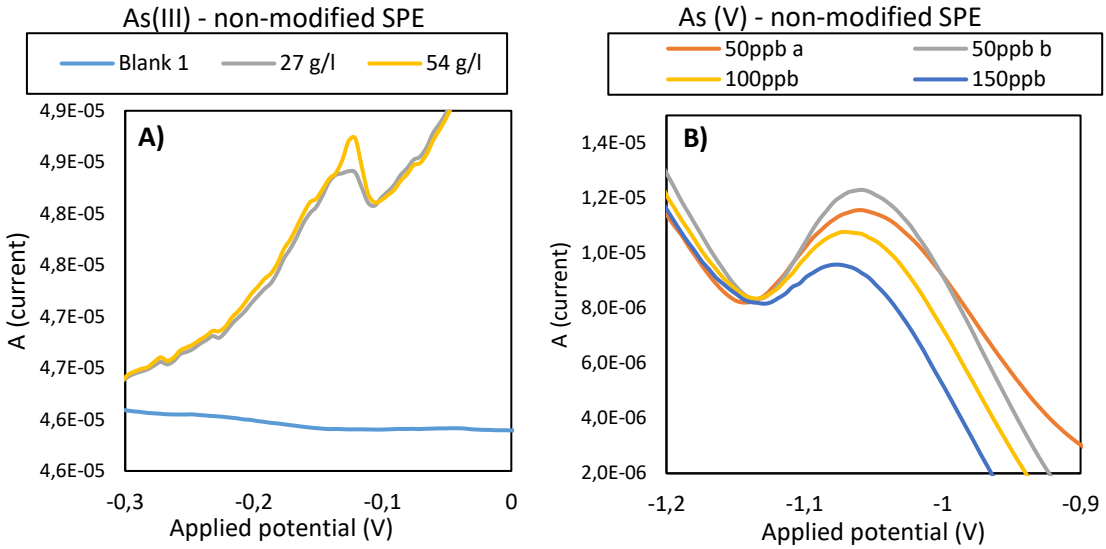


Figure 40. A) Stripping voltammetric measurements of As (III). B) As(V) with non-modified-SPCNFE at E_d = -1,3 V.

Therefore, the modification of the SPE with the AgNPs enhances the response signal as increases the repeatability and the sensibility for the detection of the metal.

5.6. Calibration curves with synthetic AgNPs

The different synthetized silver nanoparticles were used for the modification of the SPEs in order to establish which one gives better signal response for As(V) quantification. Thus, Ag-nanoseeds-SPCNFE, Ag-nanoprisms 800-SPCNFE and Ag-nanoprisms 1600-SPCNFE were prepared.

Figure 41 shows the obtained voltammograms at E_d= -1,3 V and the respective calibration curves for the different AgNPs based sensors. All of them have a good lineal correlation coefficient (greater than 0,99) up to a maximum concentration when the electrode surface is saturated with arsenic. Because of that, calibration curves are only represented until the concentration of saturation, which maximum was 50 ppb for the Ag-nanoseeds. This saturation can also be observed in the voltammogram as the nanoseeds have crescent peaks whereas the nanoprism reach a point where the peaks quickly overlap.

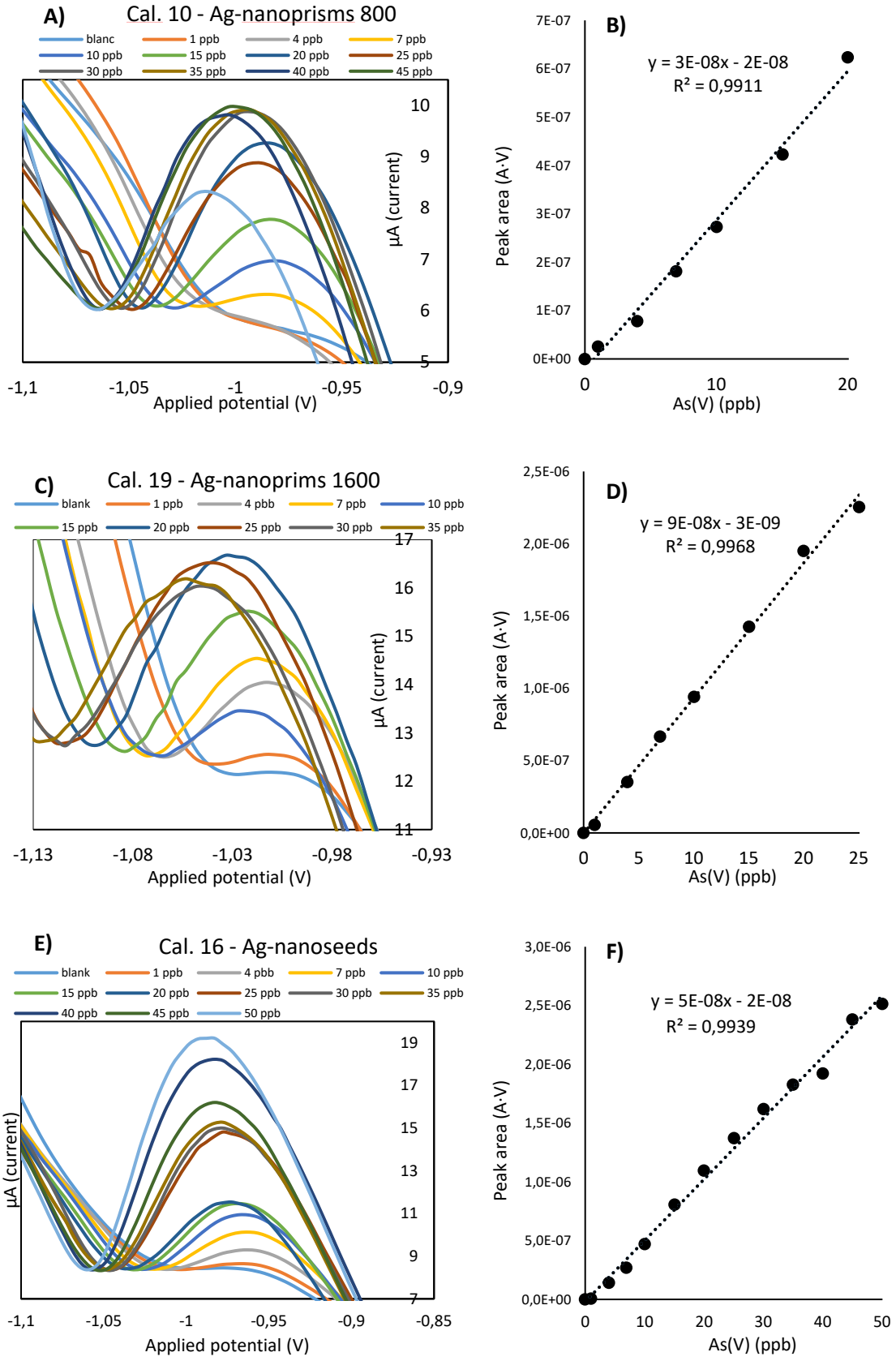


Figure 41. Voltammograms and calibration curves obtained using Ag-nanoseeds-SPCNFE(A,B), Ag-nanoprisms 800-SPCNFE(B,C) and Ag-nanoprisms1600-SPCNFE(D,E) for the determination of As(V).

The calibration parameters are reported in Table 5. The best LOD and LOQ are for Ag-nanoprism 1600 but the linear range is much more wider for the Ag-nanoseeds. In fact, if the calibration parameters are also calculated up to 25 ppb for the Ag-nanoseeds, the LOD is practically as low as for the nanoprisms, making them the best candidate. Furthermore, nanoseeds seems better option than Ag-nanoprisms 800 as the LODs are similar for a similar range, and the slope, which is also considered the sensitivity, is much better for the Ag-nanoseeds than the Ag-nanoprisms 800.

Table 5. Calibration parameters for determination of As(V) with SPCNF modified with the synthetized Ag nanoparticles.

Nanoparticles	LOD (ppb)	LOQ (ppb)	R^2	Slope (nA·U/ppb)	Lineal range
Ag-nanoprisms 800	2,30	7,66	0,9911	30,8	8-20 ppb
Ag-nanoprisms 1600	0,64	2,13	0,9993	93,4	2- 25 ppb
Ag-nanoseeds	4,23	14,10	0,9939	52,0	14-50 ppb
Ag-nanoseeds	1,35	4,51	0,9979	86	1-25 ppb

The morphology of the nanoparticles seems to be determinant in the saturation concentration of arsenic in the electrode since the nanoseeds (whose linear range is quite greater) are spherical and therefore, they provide a greater surface area of contact with the solution. In the other hand, nanoprism have triangular shape.

Ag-nanoprisms 800 seems to be the worst candidate, as nanoseeds and nanoprisms 1600 are fairly similar. This could be attributed again to the morphology of them. The number 800 and 1600 corresponds to the µl of seeds that where added before the nanoprisms growth, meaning that Ag-nanoprisms 1600 are more similar to nanoseeds as they may have nanoparticles which did not grow and remained spherical.

Repetitions of measurements with Ag-nanoseeds were made, obtaining in all the cases a correlation coefficient higher than 0,99, and the lowest LOD achieved was 1,35 ppb for a lineal range up to 25 ppb and the lowest LOD up to 50 ppb range was 1,66 ppb. The highest LOD was 4,23 ppb (Table 6). The calibration curves are represented in Annex (Figure 55).

Table 6. Calibration parameters of repetitions for determination of As(V) with SPCNF modified with the synthetized Ag-nanoseed nanoparticles.

	LOD (ppb)	LOQ (ppb)	R^2	Slope (nA·U/ppb)	Lineal range
Cal 16	4,23	14,10	0,9939	52	1-50 ppb
Cal 23	1,35	4,51	0,9979	86	1-25 ppb
Cal 26	3,69	12,29	0,9954	112	1-50 ppb
Cal 27	1,58	5,26	0,9971	20	1-25 ppb
Cal 28	1,66	5,54	0,9991	27	1-50 ppb

5.7. Calibration curves with "green AgNPs"

The "green" synthetized AgNPs (via "green" sources) were synthesized by the Department of Chemical Engineering at the University of Girona, in collaboration with a larger project. They provided various type of AgNPs, with two different pH, 4 and 6, and with different washes (2 and 3 filtrates).

In a first experiment with AgNPs pH = 4 and 3rd wash, a great difference was observed compared to the synthetic ones, since the arsenic peak was much more attenuated and elongated (Figure 42). The LOD and the LOQ are also slightly worse compared to the synthetic ones (Table 7).

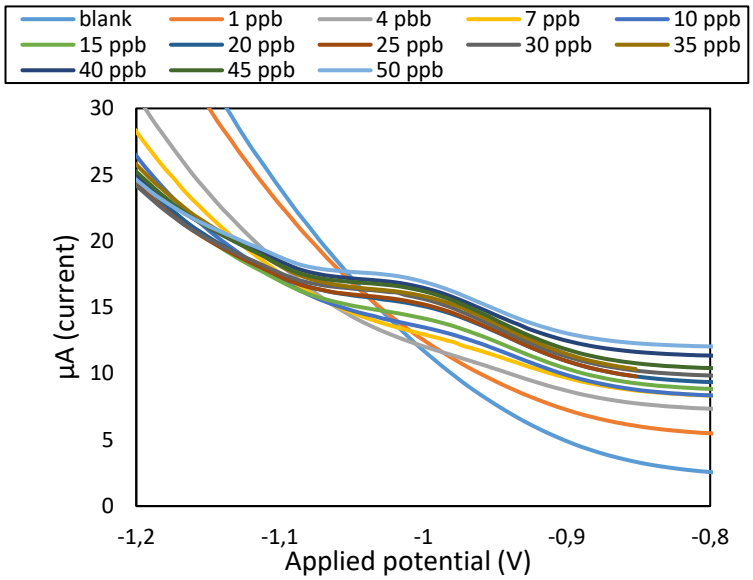


Figure 42. Stripping voltammetric measurements of As(V) with range concentrations of 1 to 150 ppb in a solution of HCl (pH = 2) using an E_d of −1,3 V during a t_d of 120s on green Ag-NPs pH = 4, 3rd wash.

Table 7. Calibration parameters for determination of As(V) with SPCNF modified with green Ag-NPs pH = 4, 3rd wash.

Nanoparticles	LOD (ppb)	LOQ (ppb)	R^2	Slope (nA·U/ppb)	Lineal range
Green NPs - pH = 4, 3rd wash	2,41	8,03	0,9956	11,67	10-30 ppb

The main cause of this fact could be the low concentration of silver that the provided solutions containing the NPs could have. That is why the E_f was extended to be able to see the silver peak (known to appear at 0,1 V). In Figure 43 it can be observed that the first peak, higher and more pronounced, is silver, and the second at 0,15 V, is the ascorbic, which concentration was increased to distinguish what peak was each.

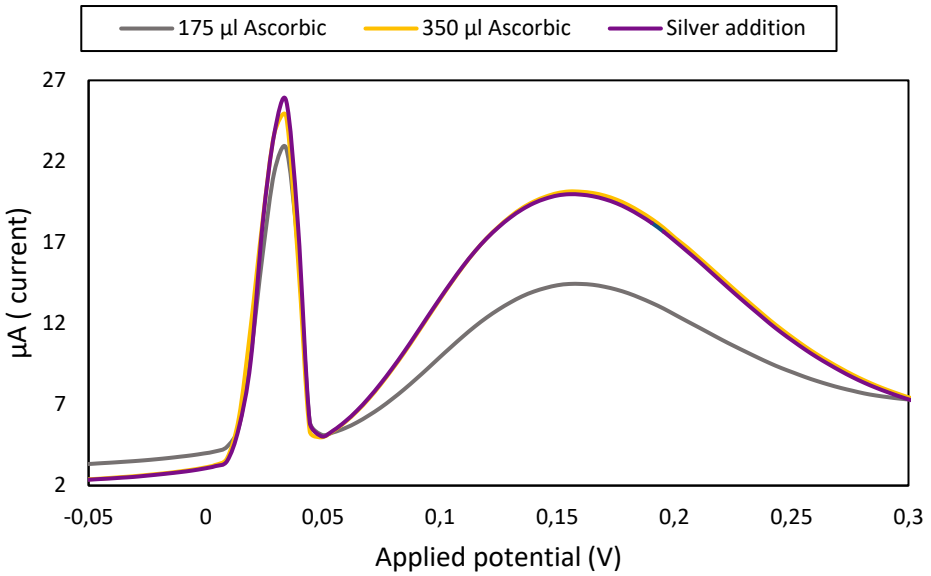


Figure 43. Stripping measurements with "green" Ag-NPs pH=4, 2nd wash. Observation of silver peak and ascorbic acid peak.

In contrast, for nanoparticles of pH = 6, the peak of arsenic was almost inexistent and the silver peak was much lower than before (Figure 44).

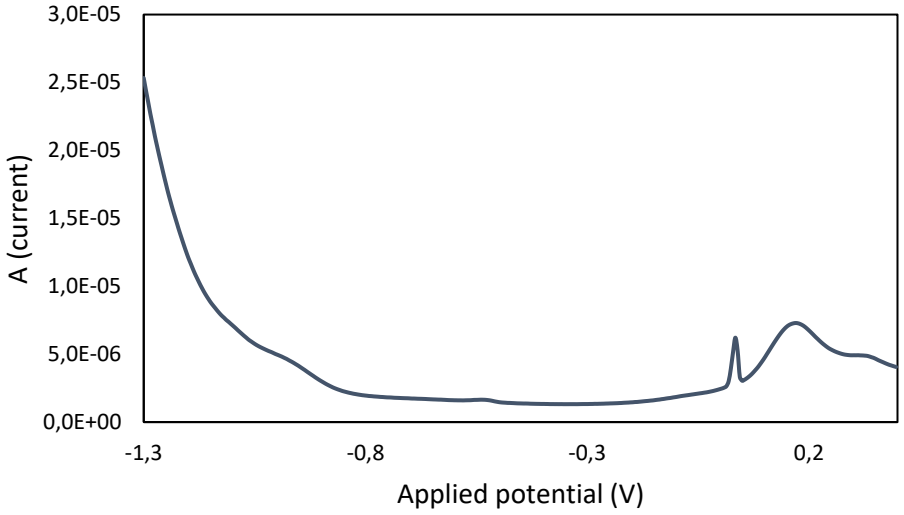


Figure 44. Stripping measurements with "green" Ag-NPs pH=6, 2nd wash at 140 ppb concentration of As(V).

To increase the concentration of silver, 3 layers of nanoparticles were deposited on the SPE (the same previous procedure of drop casting but 3 repetitions and with 20µl of NPs each time). Only a similar peak to that achieved with synthetic NPs was obtained until the concentration of arsenic was considerably increased (up to 400 ppb) (Figure 45).

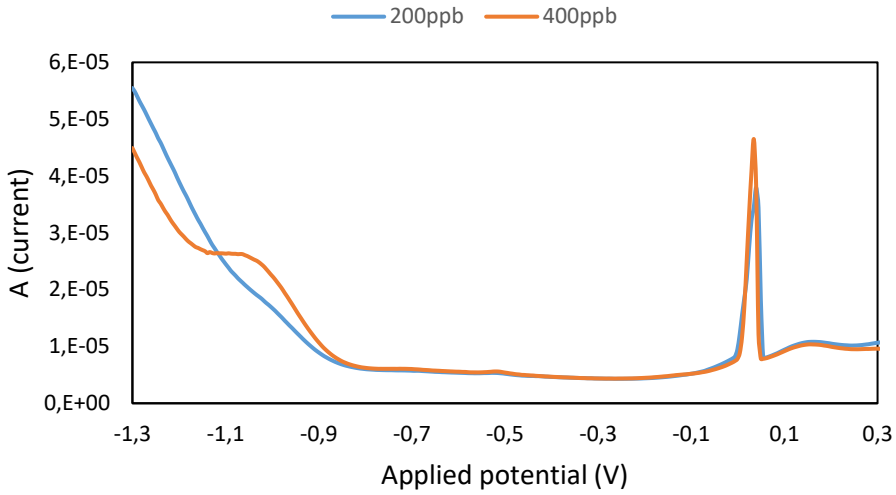


Figure 45. Stripping measurements with 3 layers of "green" Ag-NPs pH=4, 2nd on SPE for the determination of As(V).

It was decided to test the effectiveness of these nanoparticles for the detection of other ion metals with which the UB's electrochemistry group had already worked, which are Pb(II) and Cd(II). The optimal E_d that they found previously was -1,4 V. The results obtained in this case are shown in Figure 46. The peaks of Pb and Cd can be observed at -0,6 and -0,5 V respectively, although they are split into two (also in -1 and -0,8 respectively), probably because there would be abundant areas without nanoparticles, thus the WE would be working simultaneously as silver and carbon electrode, with different signal zones. In another repetition with only Pb(II) (Figure 47), the Pb peak did not split, proving the effectiveness of nanoparticles with these metals, although the peaks were not as high or as pronounced as in previous works the synthetic AgNPs (41).

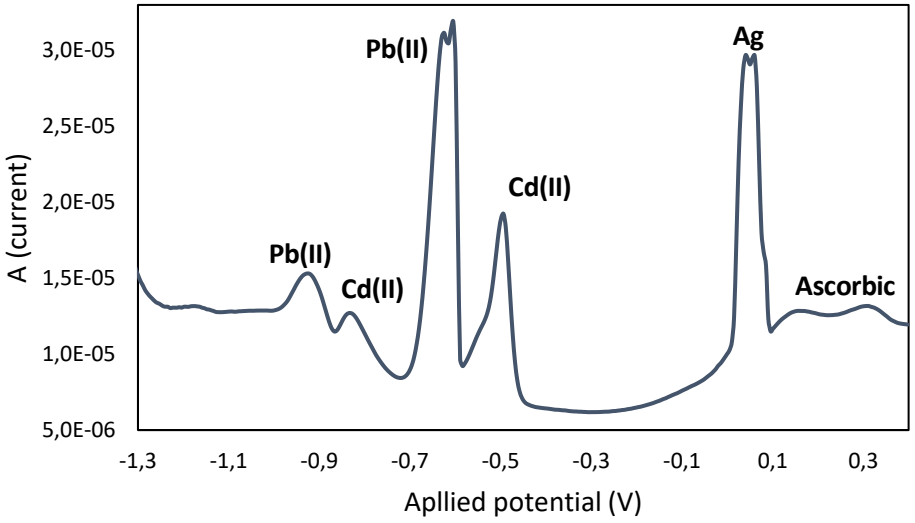


Figure 46. Stripping measurements with 3 layers of "green" Ag-NPs pH=4, 2nd on SPE for the determination of As(V) with a concentration higher than 500 ppb, Pb(II) at 148 ppb and Cd(II) at 112 ppb at E_d= -1,4 V.

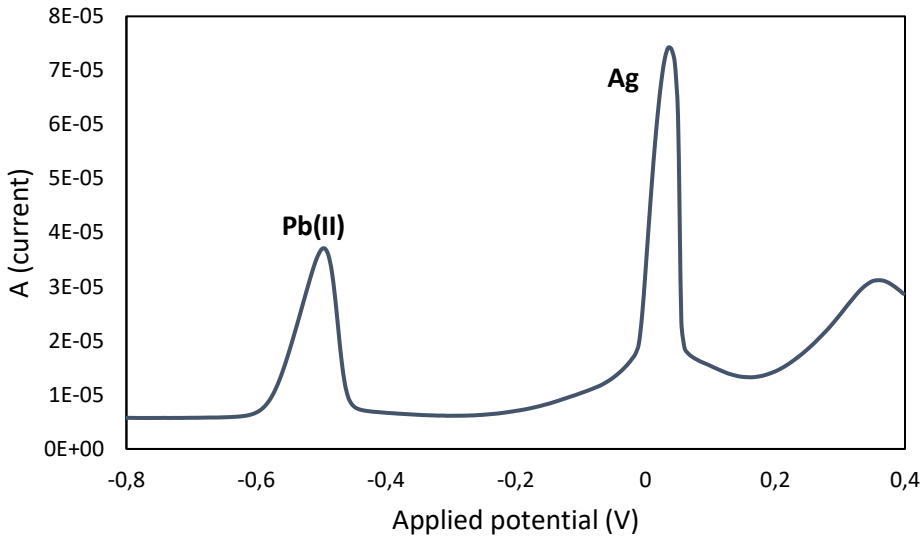


Figure 47. Stripping measurements with 3 layers of "green" Ag-NPs on SPE for the determination of Pb(II) at 148 ppb at E_d= -1,4 V.

For this reason, the synthetic NPs ones, and in particular, the nanoseeds were used to perform the real sample.

5.8. Application to a spiked sample – standard addition

Once the nanoparticles were characterized and the determination of arsenic with them was optimized, the real sample analysis was carried out, which was a "spiked" tap water, that is, tap water with the addition of standard As(V). Its concentration was determined through standard addition method.

The spiked sample was prepared by adding As(V) dilution (prepared from the standard As(V) 1000 ppm) into tap water from local water distribution network, resulting in a theoretical concentration of 1 ppm. For voltammetric determinations, 355 µl of the spiked sample was added to 35 ml of HCl (and also 175 µl of ascorbic acid) for obtaining a solution concentration of 10 ppb of As(V). Once this solution is analysed by DPASV, two more additions of As(V) standard solution are made, up to 15 and 20 ppb. However, as explained before, in the standard addition plot, the point at zero concentration added As(V) is the reading of the unknown (the spiked sample), the other points are the readings after adding increasing amounts ('spikes') of standard solution. The absolute value of the x-intercept is the concentration of As(V) in the real sample. Table 8 reports the volumes and the concentrations added for the standard addition.

Table 8. Standard As(V) volumes added and its concentrations for standard addition method.

Point	Standard volume added (µl)	Total standard volume (µl)	Total cell volume (µl)	Concentration As(V) added (ppb)
"Spiked sample"	-	0	35530	0
Addition 1	180	180	35710	5,04
Addition 2	180	360	35890	10,03

The obtained voltammogram is in Figure 48, where it can be observed that there is an arsenic peak for the spiked sample, which means that the components of tap water (which can be other metal ions as calcium and magnesium) does not seem to interfere in the quantification of the arsenic. This fact is corroborated with the calibration curves (Figure 49).

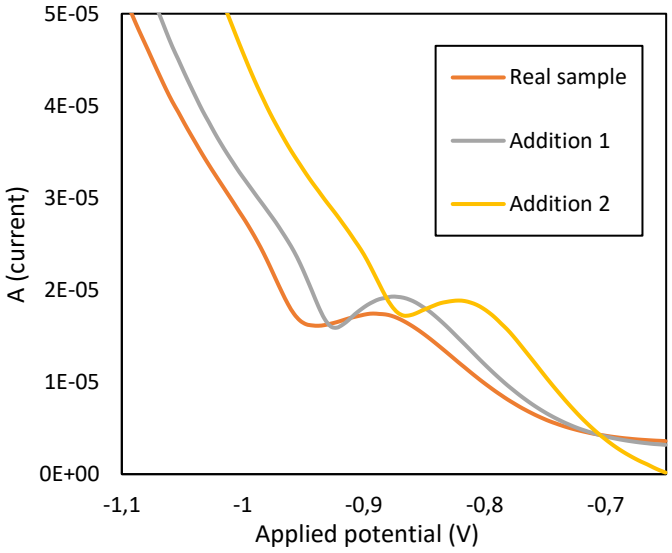


Figure 48. Stripping measurements with Ag-nanoseeds-SPCNFE for the determination of As(V) in spiked water sample.

The obtained slopes are 0,764 µA/ppb for the peak height and 0,11 µA·V/ppb for the peak area (Figure 49). The absolute value of the x-intercept is the calculated concentration of the arsenic in the spike tap water aliquot, which results in 9,90 ppb through the peak height and 8,27 ppb through the peak area, where the supposed prepared aliquot should be 10 ppb (deviations of 1% and 17% respectively).

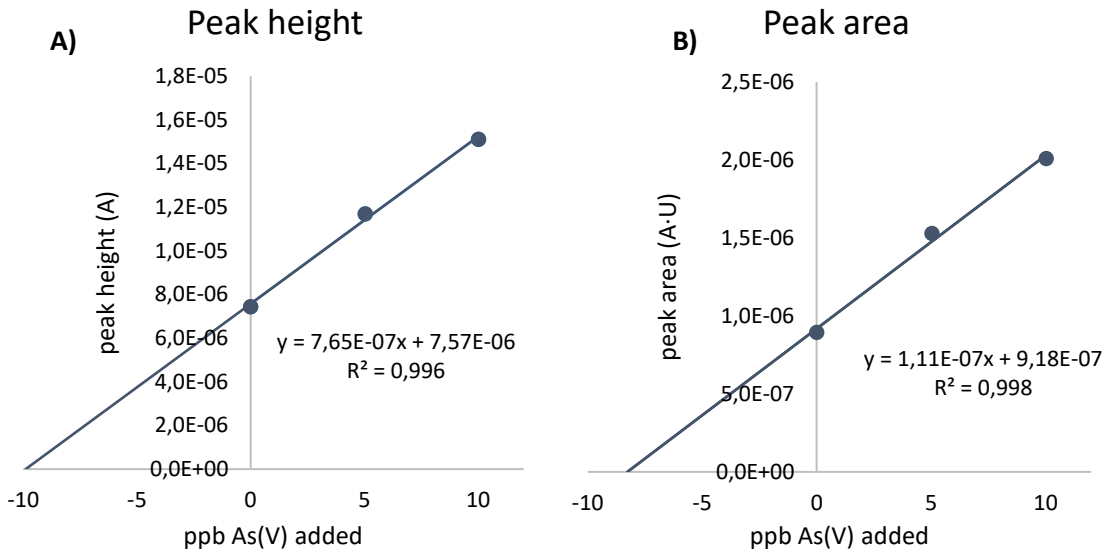


Figure 49. Peak height(A) and peak area(B) standard calibration plots for the spiked tap water sample.

6. CONCLUSIONS

The experiments performed in this work proves that AgNPs can be used for the modification of SPE to quantify As(V) in an acid solution (pH=2) and in top of that, improves the signal detection. To achieve that, two types of AgNPs were synthetized which were nanoseeds and nanoprism (800 and 1600) and were microscopically characterized with SEM. The NPs were attached to the electrode surface through drop-casting, an easy and fast method. Furthermore, the AgNPs-SPCNFE were analysed microscopically to later discuss the results.

DPASV experiments using SPE modified with the synthetized NPs showed that As(V) can be quantified, although As(III) gave more difficulties and repeatable results were not achieved. The peak of As(V) is located at -1 V and the optimal E_d was found to be -1,3 V. Besides, experiments with non-modified SPE proved that AgNPs enhances the response signal as increases the repeatability and the sensibility for the detection of the metal.

Calibration experiments were carried out for determination of As(V) with SPCNFE modified with the synthetized AgNPs. The best results were a LOD of 0,64 for nanoprisms 1600 for a lineal range up to 25 ppb. However, nanoseeds had a greater concentration of saturation, which was 50 ppb and its LOD was 1,66, similar to the above mentioned, making them the best candidate. The NPs morphology seemed to be determinant in that, since the nanoseeds are spherical and therefore, they provide a greater contact surface area with the solution.

Also “green” NPs or biosynthetic NPs were studied for the determination of As(V). However, the best results were for the synthetic ones due to probably its lower silver concentration.

Finally, a spiked tap water sample was analyzed with the Ag-nanoseeds-SPCNFE. The results indicated that tap water and its natural components did not significantly interfere in the quantification of As(V) as deviation of the calculated concentration was only 1%.

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8. ANNEX

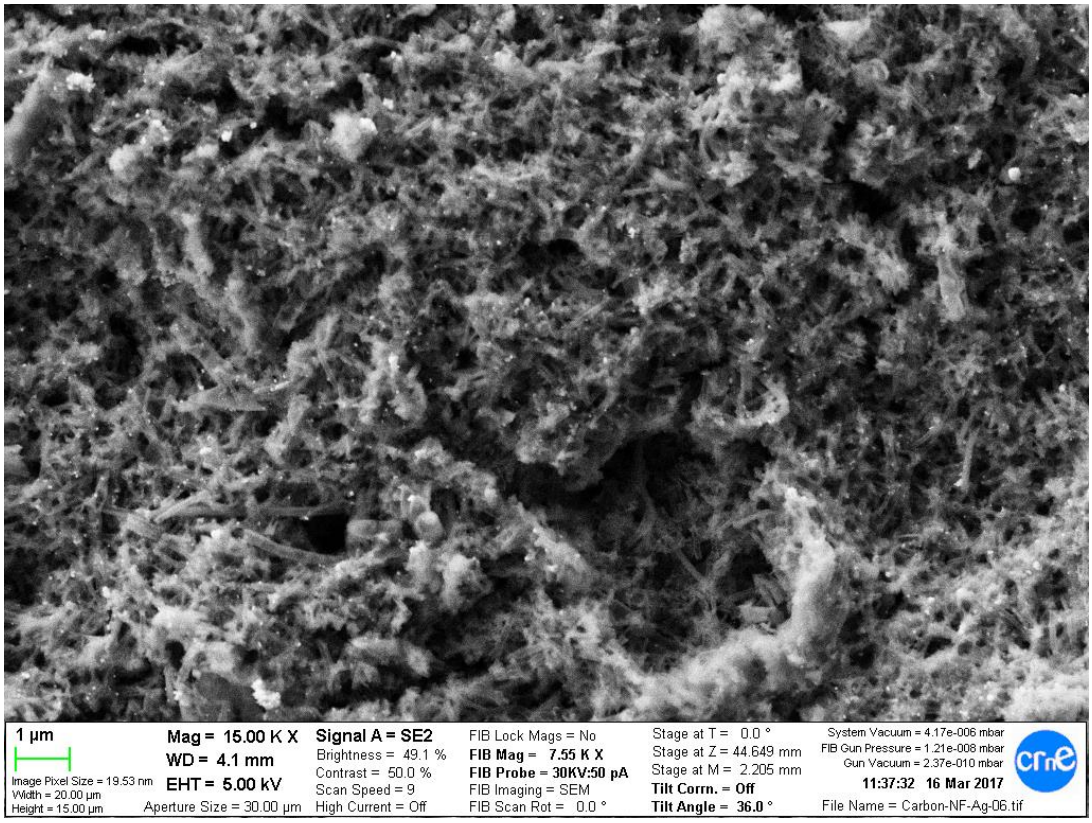

Figure 50. Magnification of SEM images of AgNPs- SPCNFE at 15k X.

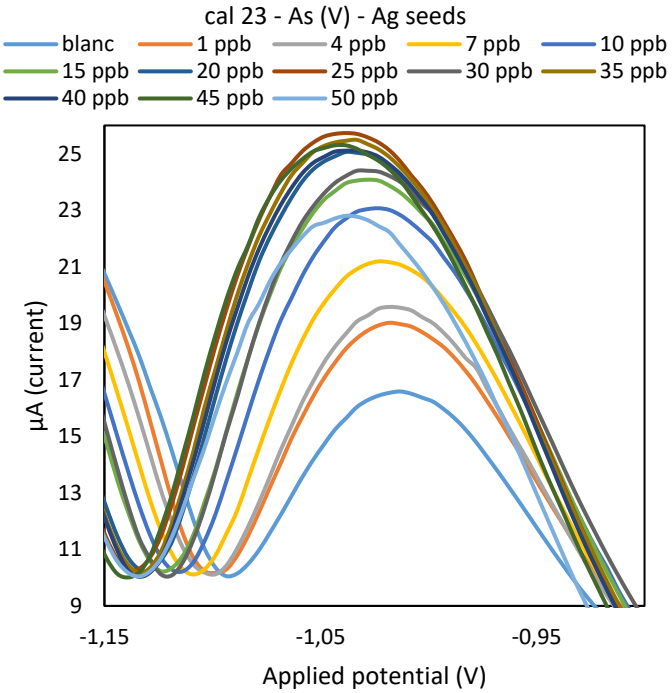

Figure 51. Voltammogram for Cal.23 for determination of As(V) with SPCNF modified with the synthetized Ag-nanoseed nanoparticles

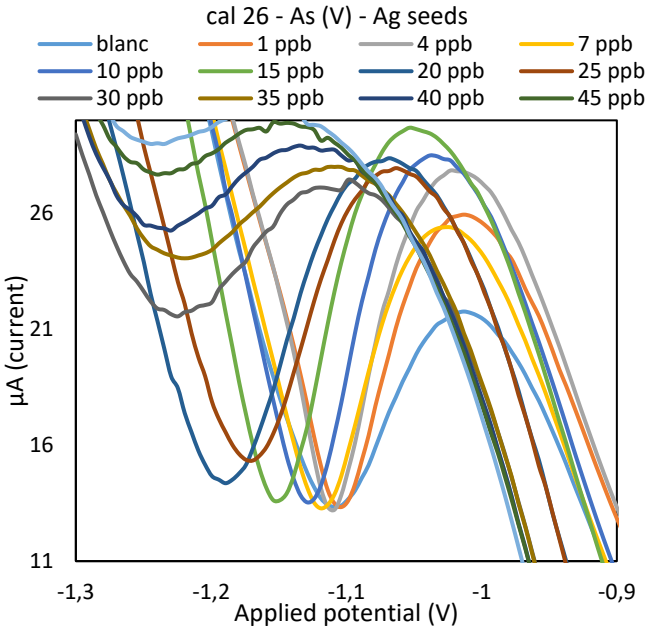

Figure 52. Voltammogram for Cal.26 for determination of As(V) with SPCNF modified with the synthetized Ag-nanoseed nanoparticles

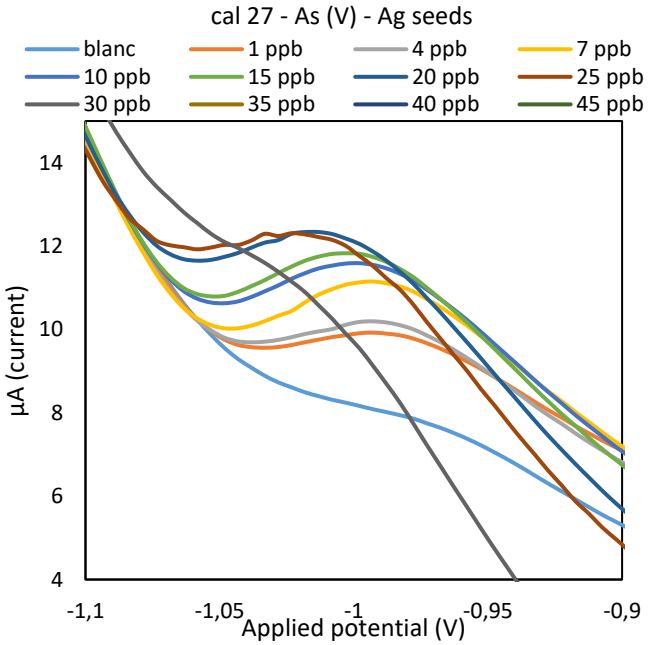

Figure 53. Voltammogram for Cal.27 for determination of As(V) with SPCNF modified with the synthetized Ag-nanoseed nanoparticles

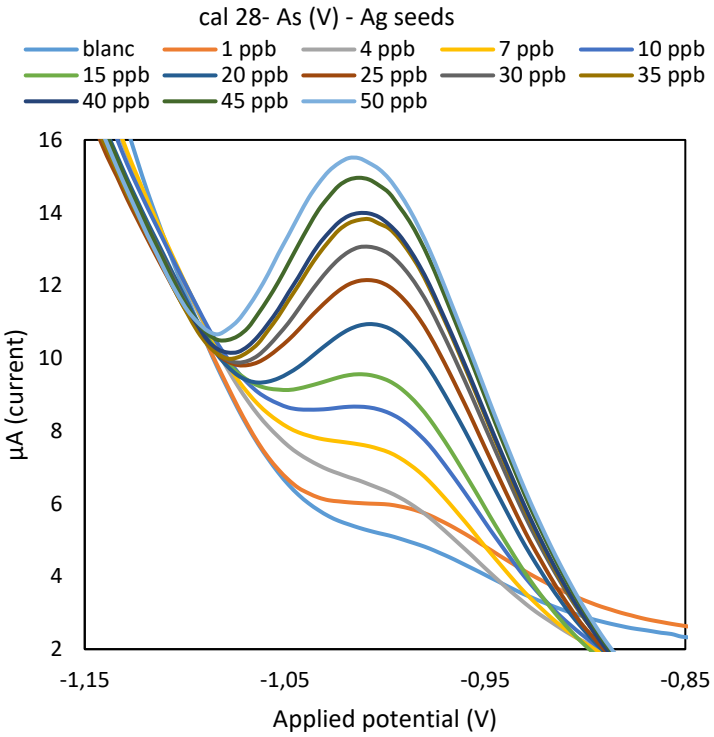

Figure 54. Voltammogram for Cal.28 for determination of As(V) with SPCNF modified with the synthetized Ag-nanoseed nanoparticles.

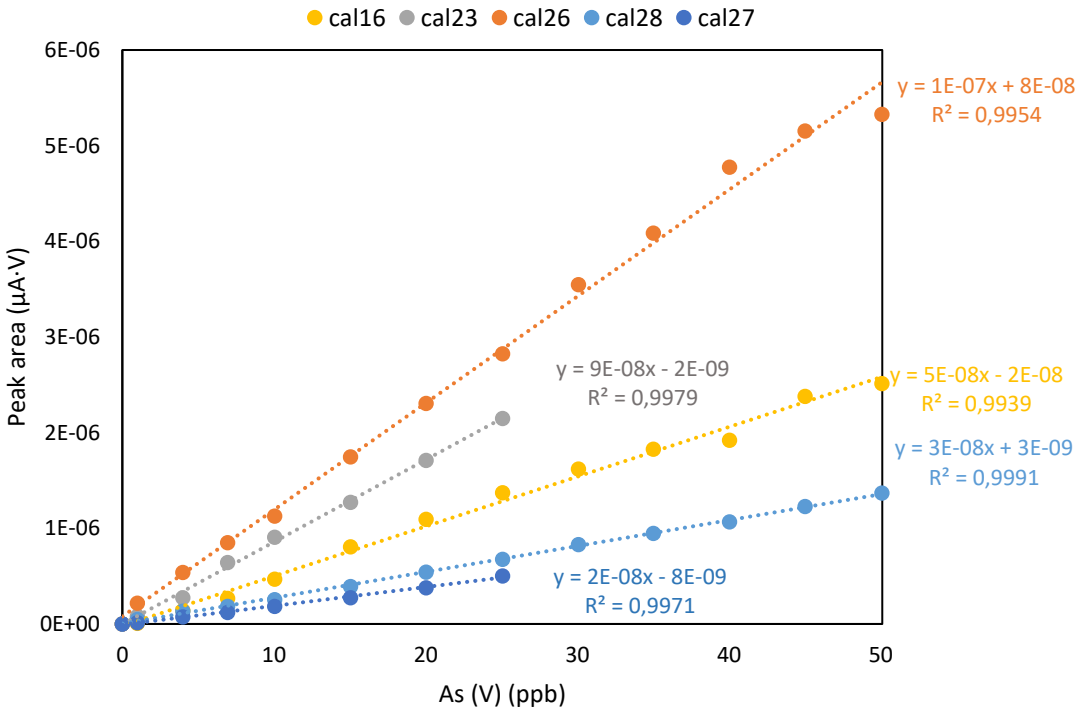

Figure 55. Peak area calibration plots of the different experiments carried out for the determination of As(V) with SPCNF modified with the synthetized Ag-nanoseed nanoparticles.