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Detection and Determination of Released Ions in the Presence of Nanoparticles: Selectivity or Strategy?

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Abstract: Metallic nanoparticles can release ionic species, but also both species can occur in the same samples. Therefore, there is a need of efficient and cost-effective methods to determine these ionic species in the presence of the corresponding nanoparticles. Electroanalytical techniques open the door to this selective detection of NPs and their ions. In this work, a methodology that allows the direct determination of ionic silver (Ag^+) in the presence of silver nanoparticles based on anodic stripping voltammetry was implemented. Silver nanoparticles were determined, after acidic digestion of the sample, by difference with respect to the total content of silver. The method was validated in terms of specific identification of silver ions, linearity, working range, limit of detection, limit of quantification, recovery, repeatability and ruggedness. All parameters are adequate for an analytical method following Eurachem recommendations. The validated method was used to determine the concentration of Ag^+ and total silver in two commercial products of colloidal silver. The results were compared with those obtained by atomic absorption spectrometry in combination with an ultrafiltration step for isolation of ionic silver. There were no significant differences in the results. The proposed methodology benefits from the intrinsic selectivity of the electroanalysis methods, allowing to eliminate the steps of pretreatments of the samples, which are necessary in other techniques. The novelty of the article lies in the direct determination of Ag (I) ions in the presence of AgNPs, without the use of previous separation steps.

Keywords: Electroanalysis · Ionic silver · Silvernanoparticles

1 Introduction

The metallic nanoparticles (NPs), which are part of products of daily use (detergents, food packaging and textiles) or biomedical objects, can evolve with their use over time, changing their surface and releasing ions [1]. Metallic ions, specifically silver ions, have a strong antibacterial effect on a range of gram-negative and gram-positive bacteria, which alter their population in external matrixes, e.g. water, external fluids [2]. Therefore, it is necessary to develop detection and characterization schemes of the species that could be generated during the aging of the products. In general, the detection of ions in the presence of the nanoparticles that generate them is relatively complicated and usually present interference problems [3]. For instance, the procedures used for the determination of silver in environmental samples, e.g. atomic absorption spectroscopy [4], neutron activation analysis [5], and inductively coupled plasma-mass spectroscopy [6], are based on the measurement of the total amount of silver, regardless of the actual silver species present in the sample. Nevertheless, modern electroanalytical techniques open the door to the selective detection of NPs and their corresponding ions even when they are both together in the same solution and avoiding the use of separation techniques prior to their determination.

Stripping voltammetry is perhaps the best technique currently available for direct measurement of trace metal ions in environmental samples [7]. The general procedure involves pre-concentration of the analyte on the electrode surface followed by the stripping step, where the analyte "deposit" dissolves back in solution because of a potential ramp. The latter step generates the electrochemical signal from which the species may be identified and quantified. This step is essential in the silver determination because silver deposits are highly stables and in many cases two electrochemical signals are obtained, this fact hinders the exact quantification of the silver ions. Several methods have been described in which acid solutions of different electrolytes are used to improve the electro-dissolution [8,9].

The detection of silver via anodic stripping voltammetry has attracted significant attention over the past decades, by means of working with different types of electrodes. Due to its low toxicity and relatively low cost, carbon has always been an attractive material for electrodes in electroanalysis. Silver has been detected with a wide range of carbon electrodes [10] but only a limited number of articles have been published on the use of glassy carbon electrodes, most of them chemically modified [11,12]. Numerous articles discuss the determination

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of silver by electrochemical techniques [12–17]. However, none addresses the direct detection and quantification of ionic silver (Ag^+) in presence of silver nanoparticles (AgNP) using glassy carbon electrodes and its application in real samples containing colloidal and ionic silver.

In the present article we propose and validate a square-wave anodic stripping voltammetry method for the direct determination of Ag^+ in presence of AgNP. This method was applied to real samples and the results compared to those obtained by conventional techniques. To the best of our knowledge, this research accounts for the first report using square-wave anodic stripping voltammetry as a novel method to determine Ag^+ in presence of the corresponding AgNP in commercial healthcare products.

2 Experimental Section

2.1 Chemicals

All chemicals in this work were purchased from Sigma-Aldrich. Silver standard solutions were prepared daily by dilutions of a 1000 mg L^{-1} stock solution. The silver nanoparticles suspensions were prepared daily by dilution of a 20 mg L^{-1} suspension. All solutions were made using ultrapure water of resistivity 18.2 μΩ (Millipore) at 25 °C.

The electrolyte solutions used were 0.1 M sodium perchlorate monohydrated.

2.2 Methods

2.2.1 Determination of Ag by Square-wave Anodic Stripping Voltammetry (SWASV)

The total Ag and Ag^+ were determined by SWASV. These determinations were carried out after dilution with $NaClO_4$ (0.1 M) to reach a concentration within the working range. For the determination of total Ag, the method was the same but preceded by the digestion of the samples for 2 h with the same volume of concentrated HNO_3. Voltammetric measurements were carried out using an AutoLab PGSTAT-12 potentiostat (Utrecht, The Netherlands). The potentiostat was operated using Autolab 2.1 software (Utrecht, The Netherlands). The auxiliary electrode was a platinum electrode, while the reference electrode was a Ag/AgCl electrode (BASi, USA). A glassy carbon electrode (GC, diameter 3 mm) also was purchased from BASi and used as a working electrode.

The electrolyte solutions used were 0.1 M sodium perchlorate monohydrated, pH 5.25. Samples were degassed thoroughly with N_2 before each measurement. The glassy carbon electrode was polished with alumina, rinsed profusely with deionized water, sonicated in water for three minutes and rinsed again with deionized water. All glassware was cleaned with 50 % nitric acid and stored in 1 % nitric acid solution when not being used.

2.2.2 Determination of Ag Using Atomic Absorption Spectroscopy (AAS)

The total Ag and Ag^+ were confirmed by AAS using a flame atomic absorption spectrometer Perkin Elmer, AAnalysts 200 (Massachusetts, USA) equipped with a silver hollow-cathode-lamp operating at 2 mA and at a wavelength of 328.1 nm. For the determination of total Ag, the method was the same as describe above. While Ag^+ determination by AAS was accomplished after an ultrafiltration step of the sample. Nanosep Pall centrifugal ultrafilter devices with cut-off membranes of 3 kDa (equivalent to a 2 nm hydrodynamic diameter). were used for Ag^+ separation. Ultrafilter devices were washed by centrifugation with 500 μL of ultrapure water prior to use. 500 μL of sample were subjected to centrifugation for 10 min at 9000 rpm and 20 °C (Thermo Heraeus Multifuge X1R, equipped with a fixed angle rotor for Eppendorf tubes, Walthman, USA).

2.2.3 UV-visible Spectrophotometry

The presence of AgNP in the samples was monitored by recording the surface plasmon resonance (SPR) band using a UV-visible double beam spectrophotometer (Jasco V-730, Japan) operated between 500 to 300 nm at a resolution of 1 nm. The standards containing nanoparticles of 40 nm were prepared at a concentration of 5 mg L^{-1}. The studied samples were diluted to a concentration of approximately 5 mg L^{-1} with ultrapure water. Standards and samples were analyzed using 3.5 mL quartz cuvettes with 10 mm of path length.

2.3 Validation

Validation was performed following EURACHEM guide [18] in terms of identification of the Ag^+, linearity, working range, limit of detection and limit of quantification, recovery, repeatability and ruggedness. The number of the replicates used for each assay was between four and ten.

Identification: To evaluated the reliability of the method, solutions of 0.5 mg L^{-1} of silver (n = 4) in 0.1 M $NaClO_4$ were used to identify the optimum conditions for Ag electrochemical detection. Silver was deposited at −0.5 V for 120 s. After an equilibration time of 5 s a single scan was carried out from 0 to 0.45 V with a step potential of 0.004 V and a scan rate of 0.04 V s^{-1}.

Repeatability: In order to evaluate the intra-assay precision, six replicates (n = 6) at two concentrations (LC: low control at 10 μg L^{-1} and HC: high control at 20 μg L^{-1}) were measured. The replicates were prepared under the same conditions and subjected to the conditions of measurements only once to avoid interference due to the possible oxidation of AgNP during the measurement process.

Linearity and working range: To evaluate linearity, three calibration curves were plotted considering the peak

area (coulombs) as a function of the concentration ($\mu g\,L^{-1}$). Four calibration solutions were prepared (2.5; 5.0; 10.0; 20.0 $\mu g\,L^{-1}$) from silver standard stock solution (n = 4). The response was linear from 2.5 up to 20 $\mu g\,L^{-1}$, lower concentrations showed a positive deviation from the calibration line. Regression analysis was performed with Origing 8.0.

Limits of detection (LOD) and quantification (LOQ): To establish the limits of detection and quantification the standard deviation of the intercept (s_a) and the slope (b) of the three calibration curves were utilized. As the method prove to be linear, the LOD (1) and LOQ (2) were expressed as: $LOD = 3s_a/b$ (1) and $LOQ = 10s_a/b$ (2).

Recovery and ruggedness: Recovery was determined by comparing the responses results obtained by AAS and ASWSV for a sample of known concentration (25 $mg\,L^{-1}$). The samples (n = 4) were quantified by AAS and then diluted adequately with $NaClO_4$ 0.1 M and quantified by ASWSV. Ruggedness was evaluated to establish if the method produces similar results when minor variations are introduced. In this case, the recovery was assessed changing the working electrode (GCE_1 and GCE_2) to analyze a solution containing 25 $mg\,L^{-1}$ of silver. The results were statistically compared using t-test.

2.4 Samples

Two commercial colloidal silver products, namely Biovedik (distributed by Alex Healthy and Natural Pvt Ltd Puducherry, India) and Wellness Colloidal Silver TM (manufactured by Source Naturals, Inc. Santa Cruz, USA) were analyzed. These products are recommended as health products intended for internal consumption or as surface sanitizers for external use. The products were kept in a dark place at room temperature until analysis. Some milliliters were taken and prepared as described in 2.2.1; 2.2.2 and 2.2.3 sections.

3 Results

In this work, we aim to develop a reliable, fast and accurate method to determine Ag ions in presence of AgNP. The determination was based on electroanalytical measurements, which is a desirable alternative due to the selective detection of NPs and their corresponding ions even when they are both together in the same solution. The method allowed to determine Ag^+ and total silver in samples containing colloidal silver. The presence of AgNPs was confirmed by the detection of the SPR bands in the spectrophotometric analysis. The estimation of the concentration of the nanoparticles in the samples was achieved by determining the difference between the total silver and the ionic silver concentrations.

3.1 Validation

Identification: Figure 1 shows typical anodic voltammograms for Ag(I) in the presence of AgNPs and the supporting electrolyte ($NaClO_4$ 0.1 M). Using the instrumental parameters listed in 2.3 it was possible to obtain a single peak at 0.240 ± 0.008 V. The differences between the baseline of each measurement is due to the differences between the compositions of both samples. Other articles showed differences in baseline levels in the blank and standard [9]. Conditions in which two signals were obtained were discarded. The multiple signals may be related to having silver deposits with different crystalline structures [7].

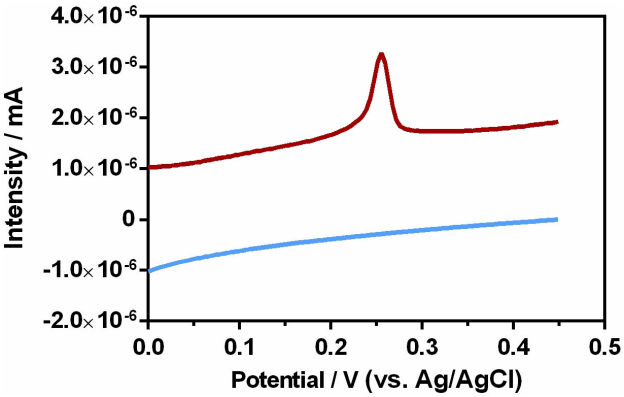

Fig. 1. SWASV of 0.05 $mg\,L^{-1}$ Ag(I) in presence of 60 nm AgNPs (0.5 $mg\,L^{-1}$) and $NaClO_4$ 0.1 M as supporting electrolyte (red). Supporting electrolyte voltammogram $NaClO_4$ 0.1 M as negative control (blue). Ag(I) was deposited at −0.5 V for 120 s. After an equilibration time of 5 s a single scan was carried out from 0 to 0.45 V with a step potential of 0.004 V and a scan rate of 0.04 $V\,s^{-1}$.

Repeatability: The relative standard deviations (RSD) were 13.64 % for HC and 8.54 % for LC (Table 1).

Linearity and working range: The regression equation for the results and its correlation coefficient are showed in the Table 1, which describe a suitable correlation for an analytical method. The method was linear in the studied range, see also Figure 2.

Limit of detection (LOD) and quantification (LOQ): The values obtained for LOD and LOQ with the used

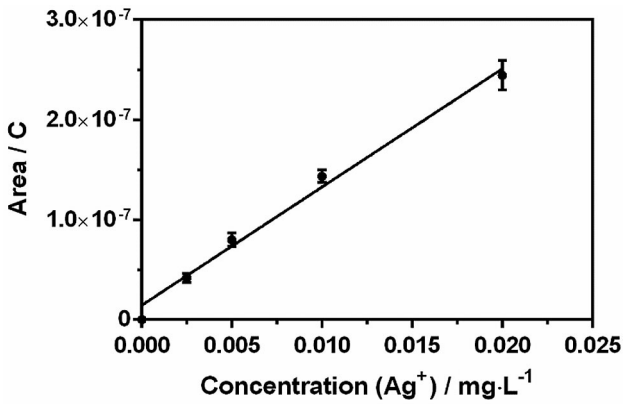

Fig. 2. SWASV calibration for Ag^+ in $NaClO_4$ 0.1 M

Table 1. Repeatability, linearity, working range, recovery, ruggedness, limit of detection and quantification of the anodic square-wave stripping voltammetry method.

Parameter	Description	Value
Repeatability	20 $\mu g L^{-1}$ (n = 6)	13.6 %
	10 $\mu g L^{-1}$ (n = 6)	8.5 %
Linearity	$R^2 = 0.9985$	$y = 1.3\ 10^{-5}x + 6.9\ 10^{-9}$
Working range		2.5–20 $\mu g L^{-1}$
Limit of detection	$LOD = 3s_a/b$	1.3 $\mu g L^{-1}$
Limit of quantification	$LOQ = 10s_a/b$	4.2 $\mu g L^{-1}$
Ruggedness	*t* test with two differente GC working electrodes	no significant differences (95 % confidende level)
Recovery	25 $mg L^{-1}$	92 ± 1%

Table 2. Concentration of Ag total and released Ag^+ in commercial samples containing colloidal silver (Biovedik and Wellness).

Product	Total Ag ($mg L^{-1}$)		Ag^+ ($mg L^{-1}$)		AgNP ($mg L^{-1}$)
	AAS	*ASWSV*	*AAS*	*ASWSV*	*Diff.*
Biovedik nominal Ag content > 25 $mg L^{-1}$	20.9 ± 0.4	20.5 ± 2.0	15.0 ± 1.0	14.7 ± 0.9	5.9 ± 0.1
Wellness nominal Ag content 30 $mg L^{-1}$	32.6 ± 1.1	32.0 ± 2.0	0.36 ± 0.01	0.4 ± 0.1	32.1 ± 0.2

method were 1.3 $\mu g L^{-1}$ and 4.2 $\mu g L^{-1}$ respectively. This limits are highly comparable with those found in the literature.

Recovery and ruggedness: Recovery was above 90 %, which is appropriate for an analytical method. The method was robust, the statistical analysis did not report significant differences at a confidence level of 95 % (Table 1).

3.2 Analysis of commercial samples

The commercial products tested were advertised to contain some form of silver (colloidal, hydro-sol or ionic) and the concentrations reported by the manufacturer are listed in Table 2. The validated method was used to determine the concentration of Ag^+ and total silver in the products by external calibration. Figure 3 shows the ASWSV voltammograms obtained from both products. The results were compared with those obtained by atomic absorption spectrometry. No significant differences were observed when a t-test (95 % a confidence level) was applied.

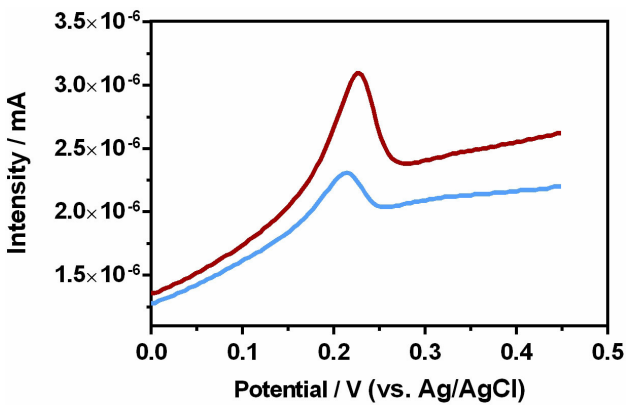

Fig. 3. SWASV of Wellness samples (red) and Biovedik sample (black) in $NaClO_4$ 0.1 M.

The measured concentrations were fairly close to the nominal contents (Table 2). The content of ionic silver in the Biovedik sample constituted around the 70 % of the total silver, while the AgNP concentration was 5.9 ± 0.1 $mg L^{-1}$. The Wellness sample contained only about 1 % of ionic silver as long as the AgNP concentration was 32.1 ± 0.2 $mg L^{-1}$. The method was effective for the determination of ionic silver in both samples, regardless ionic silver was a major or trace component.

Spectrophotometric analysis confirmed the presence of AgNP in the samples. The spectrum of both samples showed the typical SPR band of AgNP. Figure 4 shows the spectrums of Biovedik and Wellness samples together with the spectrum of AgNP standard solution (40 nm) at a concentration of 5 $mg L^{-1}$. The samples Biovedik and Wellness exhibited strong absorption at 422 and 412 nm, respectively, which confirms the presence of AgNP. The difference between the intensities of the spectra indicates that the concentration of nanoparticles in the Wellness sample is higher than in the Biovedik sample. This fact is in agreement with the result of the quantification by our electroanalytical method. The estimated concentration of AgNP in the samples was calculate by difference between total Ag and Ag^+. Table 2 shows the mean concentration of AgNP in the commercial samples, calculated by averaging the result of the quantification by AAS and ASWSV methods.

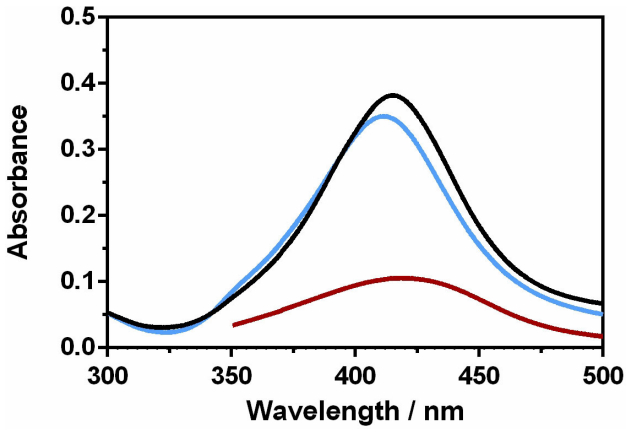

Fig. 4. Ultraviolet-visible absorption spectrum of a standard AgNP solution of 40 nm (5 mg L^{-1}) (black), Wellness (red) and Biovedik sample (blue).

4 Discussion

In this work, we addressed the voltammetric determination of Ag^+ in real samples. The obtained results demonstrate that we could efficiently and in a reproducible manner determine Ag^+ in presence of AgNP. To the best of our knowledge there are no reports on using this methodology, which makes it a valuable alternative for the reliable determination of ions in the presence of its metallic nanoparticles. However, due to the lack of literature addressing this problem, it is difficult to establish a proper comparison. The results of the validation were in agreement with what has been published for methods determining silver in other systems (other working electrodes or modified electrodes) [19,20–22]. In this work, Ag^+ was properly identified and the achievement of a single signal was possible. Some authors refer the presence of multiple peaks in the voltammogram as a consequence of the deposition of silver with different crystalline structures [7]. In our opinion we obtained a single peak signal because of the extensive cleaning step of the glassy carbon electrode (50 % HNO_3) before each measurement, making sure that the surface of the electrode was homogeneously clean for the new deposition. The non-modification of the surface of the electrode is an advantage of the proposed method, reducing the preparatory steps in comparison with other methods and hence the analysis time and the sources of uncertainty, as well as the number of reagents needed.

The repeatability was 13.6 % for HC and 8.5 % for LC, which are in accordance with values adopted in the validation guides of bioanalytical methods, which allows a variation of up to 25 % in the lower control and up to 15 % in the upper control [23]. Values obtained were also in agreement with other electroanalytical methods which present about 10 % variability [7,9].

The linearity of methods for Ag^+ detection have been reported within working ranges similar to ours [9,19].

The common procedures for the calculation of LOD and LOQ as three and ten times the standard deviation of a blank are not well-suited for electroanalytical techniques that exhibit a low background noise, in this case it is preferable to use the standard deviation of the intercept of the calibration curve [24]. The LOD and LOQ achieved in our case, are highly comparable with values already reported, in the literature it has been found higher [8,4] and similar limits [7,9,13,17,20]. This allows the application of the method to samples with low concentration of ionic Ag.

Recoveries between 70 to 130 % have been reported previously in the literature [10,11]. Our results are within this range, being 92 % a recovery value suitable for analytical methods. Ruggedness is defined as measure of method capacity to remain unaffected by small, but deliberate variations in method parameters, providing an indication of the method reliability during normal usage [18]. Although we could not find comparable values in the literature for the evaluation of this parameter, the present method can be considered robust because there were no significant differences in the recovery of Ag^+ when measurements were done with two different glassy carbon electrodes.

The determination and quantification of silver ions in commercial products were achieved successfully. The measured concentrations were fairly close to the nominal Ag contents (Table 2). The content of ionic silver in Biovedik sample constituted around the 70 % of the ionic silver and the AgNP concentration was 5.9 ± 0.1 mg L^{-1}, while the Wellness sample contained only about one percent of ionic silver and the AgNP concentration was 32.1 ± 0.2 mg L^{-1}. The method was effective for the determination of ionic silver both when it was the main component or when present at trace level. The results obtained for Ag^+ by AAS were in close accordance with the results obtained by ASWSV. To our knowledge this is the first report which accounts for application of Ag^+ determination method in samples that contain silver nanoparticles.

5 Conclusions

Square-wave stripping voltammetry at a glassy carbon electrode is a useful method to measure Ag^+ selectively in presence of AgNP. The method showed to be selective, repeatable, robust and linear. The LOD and LOQ were 1.3 μg L^{-1} and 4.2 μg L^{-1}, respectively. The method was successfully applied to two commercially available samples which contain ionic silver and silver nanoparticles. The Biovedik sample had 20.5 ± 2.0 mg L^{-1} of total silver of them 14.7 ± 0.9 mg L^{-1} were Ag^+. In this sample most of the silver was found as Ag^+, and only 5.9 ± 0.1 mg L^{-1} were as AgNP. On the other hand, the Wellness sample was constituted by 0.4 ± 0.1 mg L^{-1} of Ag^+ and 32.1 ± 0.2 mg L^{-1} of AgNP for a total content of silver of 32.0 ± 2.0 mg L^{-1}. In both samples was possible to quantify

adequately the Ag^+ in presence of AgNP this result was verified by comparison with an alternative AAS method.

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