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Biosynthesized silver nanoparticles: electrochemical application

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In this research, a fast, simple, "green" method for synthesis of silver nanoparticles (AgNPs) using flower water extracts of *Achillea millefolium* and *Lavandula angustifolia* wastes as reducing agents was demonstrated. The morphology and phase composition of the biosynthesized AgNPs were determined by Transmission Electron Microscopy (TEM) and Selected Area Electron Diffraction (SAED). AgNPs were deposited onto a spectroscopic graphite surface, applying two different procedures, and stabilized using chitosan to build modified electrodes-catalysts for reduction of hydrogen peroxide (H_2O_2). The electrochemical performance of the modified electrodes was studied by means of cyclic voltammetry and chronoamperometry at pH 7.0 and their applicability for amperometric detection of H_2O_2 was demonstrated. The modified electrodes exhibited a rapid, sensitive and reproducible response for quantitative determination of H_2O_2 at applied potentials of –0.2 V and –0.3 V (*vs*. Ag/AgCl, 3M KCl). It was established that the electrocatalytic activity of the electrodes modified with biosynthesized AgNPs significantly depends on the nature of the used plant extract. In the target reaction (electroreduction of H_2O_2) the electrode based on AgNPs, synthesized using *Achillea millefolium* waste, possesses significantly higher catalytic activity in comparison with the modified electrode based on AgNPs, synthesized using *Lavandula angustifolia* waste.

Keywords: biosynthesis, silver nanoparticles, waste valorization, hydrogen peroxide, amperometric sensor

INTRODUCTION

Silver nanoparticles are known to have broad-spectrum and robust antimicrobial properties and have been extensively applied in medicine. In the field of electrocatalysis, due to their unique physical and chemical properties, AgNPs play an important role in improving the electrochemical detection of various analytes. A number of research groups confirmed the remarkable catalytic activity of AgNPs for H_2O_2 reduction and successfully employed AgNPs-modified electrodes as a sensing interface to construct enzyme-free H_2O_2 electrochemical sensors. Modification of the electrode surface with AgNPs has been performed to enhance the rate of electron transfer and decrease the required large overpotential – the major barrier for electrochemical determination of H_2O_2 at ordinary solid electrodes. Hydrogen peroxide is an analyte of great interest in medicine because it acts as a precursor in the formation of highly reactive and potentially harmful hydroxyl radicals and is one of the most important markers for oxidative stress. An excessive accumulation of H_2O_2 in the body causes various diseases such as cardiovascular disorders, Alzheimer's, DNA fragmentation, tissue damage and cancer. Hydrogen peroxide is also an industrially relevant analyte – it is used as an oxidizing and bleaching agent in the pharmaceutical, textile and paper industries; as a sterilizing agent in the dairy industry – addition of H_2O_2 to milk decelerates the growth of bacteria and retains the fermentation.

Different methods have been used for the synthesis of AgNPs – chemical reduction of Ag^+ in a solution [1, 2], microwave-assisted reduction [3], electrochemical methods [4 - 6], UV irradiation [7, 8], radio frequency magnetron sputtering [9] and latterly "green" synthesis [10]. The use of renewable materials, environmentally benign reducing agents and nontoxic solvents are the key points of "green" chemistry and the focus of researchers over the past decade. Recently AgNPs have been successfully eco-friendly synthesized using microorganisms (bacteria or fungi) and some plant products (*Aloe vera* plant extract, lemongrass leaves extract, green tea, neem leaf, neem kernel extract, etc.). Despite substantial progress in obtaining and applying AgNPs, the development of new methods for synthesis of AgNPs is still imperative.

To the best of our knowledge, there are no reports on the use of extracts of *Achillea millefolium* and *Lavandula angustifolia*, wastes from essential oil industry, for the synthesis of AgNPs. In this connection, the aims of the present

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work were: 1/ to explore the capability of flower water extracts of *Achillea millefolium* and *Lavandula angustifolia* wastes in precipitating AgNPs from an aqueous solution of silver nitrate, and 2/ to investigate the morphology of the so-obtained biosynthesized AgNPs, as well as their ability for amperometric quantitative detection of H_2O_2. For the electrochemical studies as a bare electrode was used graphite – inexpensive, readily available material with a wide working potential range. The graphite electrode was modified with biosynthesized AgNPs, applying two different procedures. The electroactive layer was stabilized by applying thin film of chitosan onto the modified electrode surface. Chitosan is a preferable material in designing sensors and biosensors – it is a natural linear amine-rich polysaccharide, non-toxic biocompatible polymer, distinguished by its ability to form flexible, transparent membranes with sufficient mechanical strength, high adhesive consistency and protein-binding capacity. The electrochemical behaviour of the developed four different types of graphite electrodes modified with biosynthesized AgNPs was studied and their applicability for highly sensitive quantitative amperometric detection of H_2O_2 was demonstrated. The obtained electroanalytical data were compared to the state of the art, i.e. to published electroanalytical parameters for other electrodes modified with AgNPs.

EXPERIMENTAL

Materials

The working electrode was a disc from spectroscopic graphite with visible surface area *ca.* 25 mm^2 (RWO, Ringsdorf, Germany). Na_2HPO_4 and NaH_2PO_4 (Sigma-Aldrich), $AgNO_3$, $NaNO_3$ and CH_3COOH (Merck), uric acid, ascorbic acid, citric acid, D-glucose and H_2O_2 (Fluka), chitosan (Acros) were of analytical grade and used as received. Phosphate buffer solution, 0.1 M, was prepared with monobasic and dibasic sodium phosphates dissolved in double distilled water with pH 7.0, adjusted with a pH-meter model MS 2006 (Microsyst, Bulgaria). Double distilled water was used to prepare aqueous solutions.

The *Achillea millefolium* wastes were obtained from Mirkovo distillery (Mirkovo, region of Sofia, Bulgaria, 2017 harvest). The *Lavandula angustifolia* wastes were obtained from Zelenikovo distillery (Zelenikovo, region of Plovdiv, Bulgaria, 2017 harvest). Both wastes were generated after steam distillation of the fresh biomass. The wastes were inspected, dried at 50 °C, and kept frozen until further treatment.

Extraction of wastes with distilled water was performed as follows: 150 g of dry residue were treated with 1000 mL of water for 1 h at 60 °C and left for 24 h at room temperature at constant stirring. The mass was filtered and the insoluble residue was extracted with additional 500 mL of water at the same conditions.

Apparatus and measurements

The samples of the AgNPs for TEM analysis were prepared by placing a drop of the suspension on a standard copper grid, covered by amorphous carbon layer and allowing the solvent to evaporate at room temperature. Images were acquired by high resolution transmission electron microscope JEOL JEM 2100 (JEOL, Japan) at accelerating voltage 200 kV. Statistical analysis of AgNPs size distribution was carried out with Image J software. The identification of the phase composition of the samples was achieved using PDF-2 Database of the International Center for Diffraction Data (ICDD).

All the electrochemical measurements were performed using potentiostat EmStat3 (PalmSens BV, The Netherlands) interfaced with a computer and controlled by 'PSTrace 2.5.2' software. A conventional thermostated three-electrode cell including a working electrode, an Ag/AgCl (3 M KCl) as a reference electrode, and a platinum wire as a counter electrode was used. All the electrochemical measurements were carried out in 0.1 M phosphate buffer solution (PBS) at temperature of 25 °C. Before the measurement the background electrolyte was purged with pure argon for 15-20 min to remove dissolved oxygen.

The modified electrodes were investigated using cyclic voltammetry (CV) and amperometry at a constant potential. Amperometric (*i*-*t*) curves were registered at a constant applied potential under hydrodynamic condition. The H_2O_2 stock solution was freshly prepared before each measurement. The experimental data analysis was performed using software package 'OriginPro 8.0'.

Biosynthesis of AgNPs

Two mL of aqueous extract (*Achillea millefolium* or *Lavandula angustifolia*) was added to 6 mL of 10^{-2} M $AgNO_3$ solution and the final volume was adjusted to 10 mL with double distilled water. The addition of the extract to the aqueous $AgNO_3$ solution turned the initial yellowish color to brown indicating the formation of AgNPs.

Preparation of the modified electrodes

Prior to the modification, the bare graphite electrode (Gr) was carefully polished to mirror-like finish with emery paper with decreasing particle size, rinsed, sonicated in double distilled water for

5 min and allowed to dry at room temperature. The working surface of the cleaned and polished electrode was modified using two different approaches: 1/ through dropwise addition of 40 μL of the colloidal AgNPs solution, after applying the electroactive substance, the electrode surface was dried at room temperature; 2/ under static conditions by immersing the graphite electrode in the colloidal AgNPs solution; the duration of the adsorption process was 2 hours at temperature 25 ^{o}C. In order to stabilize the electroactive layer, a thin polymer film was applied by dropping onto the electrode surface of 5 μL of 0.5 % chitosan (in a solvent 0.1 M CH_3COOH) and drying it in air. The resulting modified electrodes, developed using *Achillea millefolium* extract as a reducing agent, will be denoted as electrode type AgNPs(AM)-CS/Gr1 and type AgNPs(AM)-CS/Gr2. The electrodes, developed using *Lavandula angustifolia* extract, will be denoted as electrode type AgNPs(LA)-CS/Gr1 and type AgNPs(LA)-CS/Gr2, respectively.

RESULTS AND DISCUSSION

Transmission electron microscopy (TEM) studies

The morphologies of AgNPs synthesized with water extracts of *Achillea millefolium* and *Lavandula angustifolia* are presented in Fig. 1 a) and b), respectively. The representative SAED pattern for AgNPs is included in the same figure (Fig. 1 c). As seen, the nanoparticles grew very tiny with spherical shape. The size distribution of *Achillea millefolium* and *Lavandula angustifolia* AgNPs and their mean sizes, 2.8 nm for AM and 3.1 nm for LA, are presented in the histograms (d) and (e). The indexing of SAED pattern reveals that Ag cubic (PDF 870720) is the only phase in the composition of both samples AM and LA. Summarized data for interplanar distances and Miller indices are presented in Table 1.

Table 1. Indexing of SAED pattern

d [Å]	hkl	Phase	PDF
2.3540	111	Ag cubic	870720
1.4415	220	Ag cubic	870720
1.1671	222	Ag cubic	870720

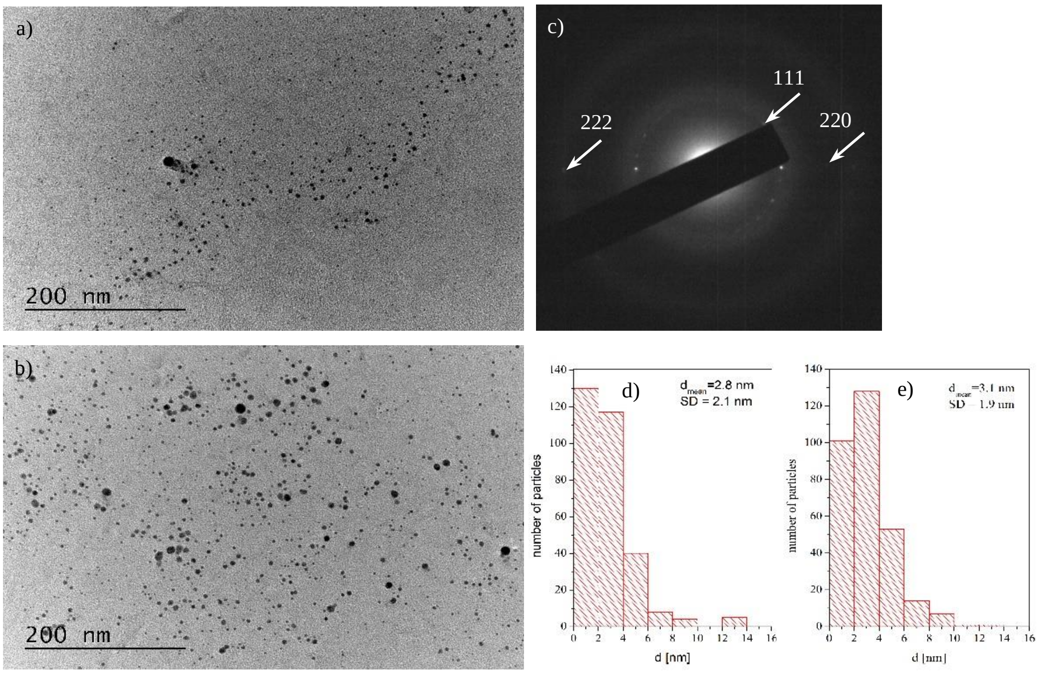


Fig. 1. TEM micrographs of AgNPs synthesized with extract of *Achillea millefolium* (a) and *Lavandula angustifolia* (b) wastes, representative SAED patterns (c) and size distribution of the corresponding particles (d) and (e).

Electrochemical studies

The presence of Ag on the surface of graphite electrodes was evidenced by cyclic voltammetry. Fig. 2 presents the cyclic voltammogram of the modified electrode type AgNPs(AM)-CS/Gr1 in deaerated 0.1 M PBS (pH 7.0) recorded in the potential range from –0.2 to 0.8 V. A pair of well-defined redox peaks was observed at 0.44 and 0.17 V, respectively, which was ascribed to the redox of silver.

In order to obtain prior information about the catalytic activity of the modified electrodes towards the electroreduction of hydrogen peroxide and to evaluate the effect of the applied potential on the current response of the electrodes, an amperometric signal was recorded in the presence of H_2O_2 at a constant potential.

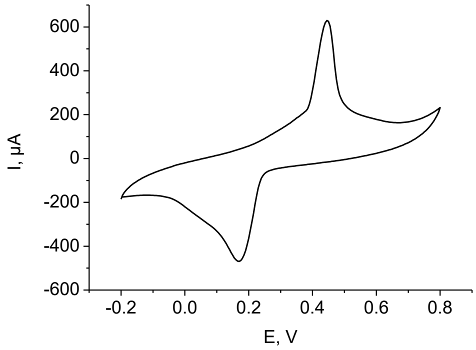


Fig. 2. Cyclic voltammogram of the modified electrode AgNPs(AM)-CS/Gr1 in 0.1 M PBS (pH 7.0); scan rate of 25 mV s^{-1}.

Fig. 3 shows the background-corrected steady-state response of the electrodes in presence of 2.3 mM H_2O_2 at applied potentials of –0.3, –0.2, –0.1 and 0.0 V, respectively. From the data presented it is evident that the catalytic activity of both electrodes increases as the polarization potential shifts negatively (for type AgNPs(AM)-CS/Gr1 the dependence was linear with a correlation coefficient of 0.994). A comparison of the current values for the two catalysts shows that electrode type AgNPs(AM)-CS/Gr1 is distinguished by a significantly higher catalytic activity than type AgNPs(AM)-CS/Gr2.

The reproducibility of the current response of the modified electrode is essential for its successful application as a sensing element. In this connection, the current signal for electrode AgNPs(AM)-CS/Gr1 (which possesses a higher response) to 2.3 mM H_2O_2 at applied potentials of –0.3 V and –0.2 V was examined. For 5 successive independent measurements at –0.3 V in presence of 2.3 mM H_2O_2 the electrode response was 271.6 ± 18.6 µA and the relative standard deviation (R.S.D.) was calculated to be 6.8 %. Analogous studies carried out at a potential of –0.2 V show electrode response of 233.4 ± 14.8 µA and R.S.D. of 6.3 %. The results prove that the modified electrode possesses satisfactory reproducibility of the current signal.

In the next step, the graphite electrodes modified with biosynthesized AgNPs were tested for amperometric quantitative detection of H_2O_2. The concentration dependence of the amperometric response of the electrodes was investigated by means of constant potential amperometry. Calibration plots for AgNPs(AM)-CS/Gr1 (Fig. 4), clearly show that the sensitivity (determined as the slope of the linear section of the calibration graph) increases and the portion of the strict linear concentration dependence of the signal is extended as the working potential shifts to the cathodic direction. The electrode sensitivity at potential of –0.3 V was found to be 1.2 times higher than the sensitivity registered at –0.2 V.

Fig. 5 shows the typical steady-state current response of the modified electrode type AgNPs(AM)-CS/Gr2 to successive additions of 0.5 mM H_2O_2 into stirred 0.1 M PBS (pH 7.0) at a constant potential of –0.3 V. Upon addition of H_2O_2 the modified electrode shows increasing reduction currents (staircase current response), corresponding to the electrochemical conversion of the analyte. The inset in Fig. 5 presents the calibration curve for H_2O_2 (14 points), built on the basis of the chronoamperometric data. The response of AgNPs(AM)-CS/Gr2 was linear up to 5.7 mM H_2O_2 ($R^2 = 0.997$).

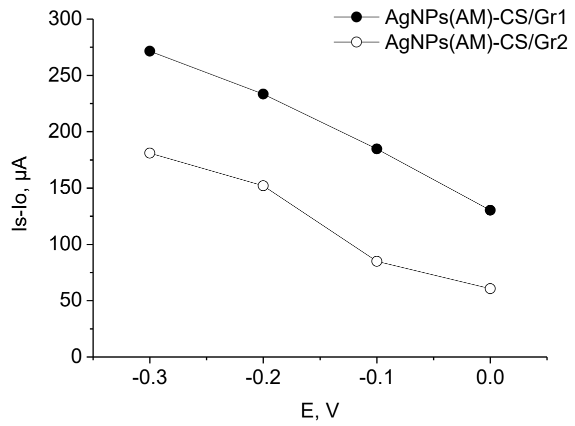
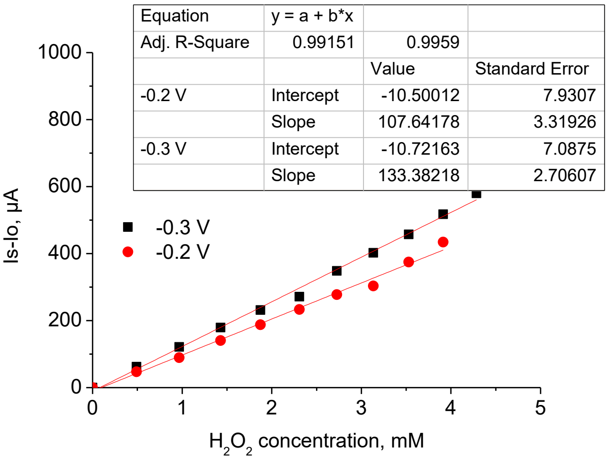


Fig. 3. Background corrected steady-state response of the modified electrodes type AgNPs(AM)-CS/Gr1 (solid circles) and type AgNPs(AM)-CS/Gr2 (open circles) in presence of 2.3 mM H_2O_2; supporting electrolyte 0.1 M PBS (pH 7.0).



Equation	y = a + b*x		
Adj. R-Square	0.99151	0.9959	
		Value	Standard Error
-0.2 V	Intercept	-10.50012	7.9307
	Slope	107.64178	3.31926
-0.3 V	Intercept	-10.72163	7.0875
	Slope	133.38218	2.70607

Fig. 4. Background-corrected steady-state response of the modified electrode AgNPs(AM)-CS/Gr1 as a function of H_2O_2 concentration; supporting electrolyte 0.1 M PBS (pH 7.0).

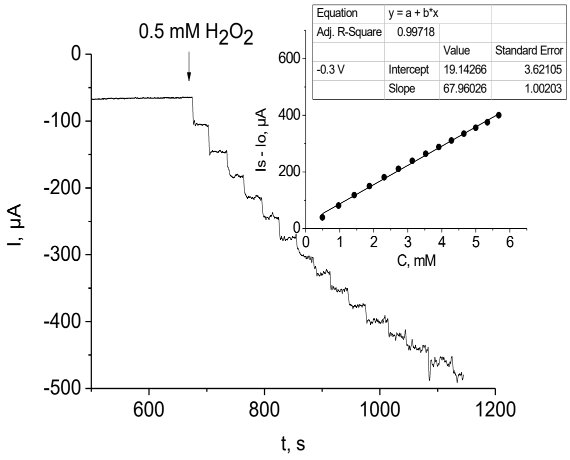


Fig. 5. Amperometric response of modified electrode AgNPs(AM)-CS/Gr2 to successive addition of 0.5 mM H_2O_2 into stirred 0.1 M PBS (pH 7.0) at an applied potential of –0.3 V; Inset: corresponding calibration plot.

Table 2 presents an overview on the main operational parameters of the electrodes modified with biosynthesized AgNPs. Concerning the analytical characteristics of the all four type modified electrodes, the graphite modified with AgNPs, biosynthesized using extract of *Lavandula angustifolia* wastes, exhibited lower activity as compared to the graphite modified with AgNPs, synthesized with extract of *Achillea millefolium* wastes.

The electrocatalytic activity of the developed electrodes also significantly depends on the modification procedure – the sensitivity of the electrode type AgNPs(AM)-CS/Gr1 was found to be about 2 times higher than this one of AgNPs(AM)-CS/Gr2 estimated under the same conditions.

Table 2. Operational parameters of graphite electrodes modified with biosynthesized AgNPs; background electrolyte 0.1 M PBS (pH 7.0).

Type of modified electrode	E, V	Sensitivity, µA $mM^{-1}cm^{-2}$	R^2	Linearity, mM
AgNPs(AM)–CS/Gr1	–0.3	533.5	0.996	4.3
	–0.2	430.6	0.992	4.0
AgNPs(AM)–CS/Gr2	–0.3	271.8	0.997	5.7
	–0.2	252.0	0.999	2.7
AgNPs(LA)–CS/Gr1	–0.3	374.7	0.999	3.5
	–0.2	304.5	0.999	3.5
AgNPs(LA)–CS/Gr2	–0.3	248.3	0.996	3.5
	–0.2	127.4	0.993	5.0

Table 3. Comparison of the operational characteristics of amperometric sensors based on AgNPs for H_2O_2 quantitative detection, with the achieved in the present work.

Sensing platform/Support electrode*	E, V	Sensitivity, µA $mM^{-1}cm^{-2}$	Linearity, M	Ref.
AgNWs/SPCE	–0.4[a]	138.4	1.42×10^{-2}	5
AgNPs/GC	–0.85[b]	–	6.5×10^{-3}	11
GR–AgNCs/GC	–0.5[a]	183.5	1.0×10^{-2}	6
Ag–NFM/GC	–0.58[a]	157	1.65×10^{-2}	9
Ag–HNTs–MnO_2/GC	–0.3[b]	11.9	4.71×10^{-3}	12
GO–AgNPs/GC	–0.3[a]	0.1218[c]	1.1×10^{-2}	13
AgNPs–PANI–HNTs/GC	–0.2[b]	74.8	4.7×10^{-3}	1
AgNPs–porous silicon/CPE	–0.45[a]	34.07[c]	5.0×10^{-3}	14
AgNPs(AM)–CS/Gr AgNPs(LA)–CS/Gr	–0.3[a]	533.5 374.7	4.3×10^{-3} 3.5×10^{-3}	Present work

[a] referred to an Ag/AgCl (3 M KCl) electrode; [b] referred to a saturated calomel electrode (SCE); [c] the unit is: µA mM^{-1}; *AgNWs – silver nanowires, SPCE – screen printed carbon electrode, GC – glassy carbon, GR – graphene, AgNCs – silver nanocrystals, NFM – nanofibrous membrane, HNTs – halloysite nanotubes, GO – graphene oxide, PANI – polyaniline, CPE – carbon paste electrode, CS – chitosan, Gr – graphite.

The experimental data indicate that the range of the strict linear dependence of the electrode signal for AgNPs(AM)-CS/Gr1 is almost the same for both applied potentials. At a potential of –0.3 V electrode type AgNPs(AM)-CS/Gr2 possesses twice as long linear portion of the calibration plot as compared to this one at potential of –0.2 V.

The performance of the here presented electrocatalysts, based on biosynthesized AgNPs, was compared with other carbonaceous electrodes modified with AgNPs. In Table 3 we have summarized various amperometric H_2O_2 sensors, with respect to the applied potential, the sensitivity and the linearity of the signal. All data presented have been published in the last 6 years. It can be seen that the proposed modified electrodes show an excellent sensitivity, several times higher than that obtained by using other carbon-based electrodes modified with AgNPs.

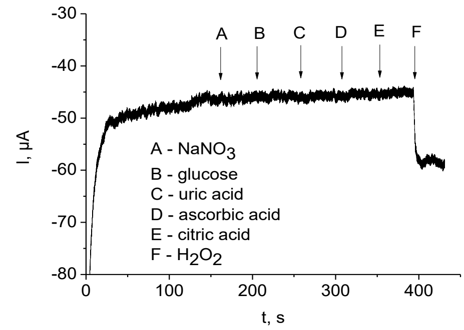


Fig. 6. Amperometric *i-t* curve of modified electrode AgNPs(LA)-CS/Gr1 for the determination of 0.15 mM H_2O_2 (F) in the presence of 3.0 mM of different interfering species, added one by one with an interval of 50 s to 0.1 M PBS (pH 7.0) at an applied potential of -0.2 V.

It is well known that some co-existing electroactive species in real samples will affect the sensor response, which could have compromised the measured value of the analyte. In order to study the selectivity of the electrodes modified with biosynthesized AgNPs, we have monitored the response of type AgNPs(LA)-CS/Gr1 in the presence of common interfering species such as $NaNO_3$, glucose, uric acid, ascorbic acid and citric acid. Amperometric response of AgNPs(LA)-CS/Gr1 towards the additions of these substances (3.0 mM), followed by H_2O_2 addition (0.15 mM), was examined at a constant potential of –0.2 V. The authentic record of the response of modified electrode (Fig. 6) clearly shows that the tested species had no effect on the H_2O_2 detection – no response was observed for modified electrode in the presence of the above mentioned substances and the current response for H_2O_2, registered after adding the substances, corresponds to the one determined in the calibration study (I (μA) = 76.13C (mM) + 2.331, $R^2 = 0.999$). These results demonstrate that the modified electrode has good selectivity for H_2O_2 and reveal the application potential of biosynthesized AgNPs for sensing of H_2O_2 in real samples.

CONCLUSIONS

Flower water extracts of *Achillea millefolium* and *Lavandula angustifolia* wastes were used as reducing agents for efficient synthesis of AgNPs, which is a novel simple approach, inexpensive and eco-friendly in nature. The graphite electrodes modified with as-obtained biosynthesized AgNPs were successfully used for reduction of H_2O_2 and their applicability for amperometric quantitative determination of H_2O_2 in micromolar concentrations was demonstrated. The modified electrodes showed a remarkable activity at applied potentials of –0.3 V and –0.2 V (*vs*. Ag/AgCl, 3 M KCl), stable amperometric response, high sensitivity in a wide linear range for H_2O_2 detection and provided a new platform for the development of electrochemical sensors.

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