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Application of carbon electrodes modified with graphene and chitosan to electrochemical sensing of ascorbate

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Graphene (G) and graphene oxide (GO) were used to modify a glassy carbon electrode (GCE) in order to enhance electron transfer. The best results were obtained when the electrode surface was modified with a thin film of GO dispersed in an aqueous chitosan solution. The resulting electrodes were characterized by cyclic voltammetry (CV) and electrochemical impedance spectroscopy (EIS). The optimized electrode was applied for the determination of ascorbate in synthetic and natural samples. The sensitivity to ascorbate is 247 ± 8 μA cm^{-2} $mmol^{-1}$ L, and the limit of detection is 13 nmol L^{-1}. The limit of detection is better than that of other sensors. The data obtained by the new method for ascorbate detection in juice were in a good agreement with the data provided by the producer.

Key words: graphene, cyclic voltammetry, atomic force microscopy, chitosan, ascorbate

INTRODUCTION

Graphene is one of the leading materials in various fields due to its unique structural, mechanical and electronic properties [1–4]. Therefore, it is applied in various fields such as medicine [5], drug delivery [6], (bio)sensing and electrochemistry [7–13], electrical energy storage devices and electric capacitors [1]. However, immobilisation of graphene on surfaces is problematic due to its tendency to aggregate in suspensions caused by the hydrophobic carbon nature. Dispersions of graphene in various solvents and polymers are widely investigated and discussed [3, 9, 10, 13–15], however, modification of graphene with various functional groups is one of the best solutions for its homogenisation [3, 13–15]. The electrochemical performance of carbon electrodes can be further enhanced and/or extended by modifying their surfaces with various materials: conducting polymers [16–18], ionic liquids [19] or nanostructured materials [20].

Chitosan (Chit) is one of such polymers used to modify graphene because it makes a homogeneous dispersion in its aqueous solution. It is a versatile biological compound obtained by alkaline treatment of chitin. It is employed in many fields allowing fabricate membranes, thin films, three dimensional structures [21] and immobilisation of graphene and carbon nanotubes [13, 22, 23]. Graphene can be dispersed in an aqueous solution of chitosan and thus cast into membranes and films that can be converted into insoluble networks [22].

Ascorbic acid (AA) is known for its multifunctionality in the body: It is important for the collagen formation, the absorption of iron by promoting its reduction, and it takes part in the immune response and in clinical cancer treatment [24]. AA is present in many foods and beverages, particularly in fresh fruits and vegetables and is widely used as a food additive and preservative [25]. Various analytical methods can be used for ascorbic acid detection [25], one of the most popular being electrochemical determination [9, 12, 26].

In the present work, the previously optimised graphene coated glassy carbon electrode [15] was applied for ascorbate

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determination. Chitosan solution was chosen as a matrix for graphene dispersion and modification to get an adhesive film on the glassy carbon electrode. Graphene concentration in dispersion was optimised in order to obtain a thin electroactive film which ensures the most effective electron transfer. Such modified electrode was tested for ascorbate oxidation and determination in modelled and natural samples. The sensitivity and limit of detection was excellent taking into account a simple sensor preparation without any additional redox compounds.

EXPERIMENTAL

Materials

Graphene flakes (8 nm height) were purchased from Graphene Supermarket (USA); $K_4Fe(CN)_6 \cdot 3H_2O$, CH_3COOH, KCl, sodium ascorbate, and chitosan from shrimp shells were obtained from Sigma Aldrich (Germany). H_2SO_4, HNO_3, $NaH_2PO_4 \cdot H_2O$, Na_2HPO_4, and NaOH were obtained from ROTH GmbH (Germany). All reagents used were of analytical grade. All solutions were prepared with ultrapure MilliQ-water (resistivity of 18.2 MΩ cm) directly taken from a Synergy 185 unit equipped with a UV lamp (Millipore, USA).

An aqueous solution of 0.5% chitosan was prepared as described elsewhere [27]. Chitosan was dissolved in aqueous 1% CH_3COOH solution; then pH was adjusted to pH 5.0 with 20% NaOH solution. Then graphene or GO was added to the solution and sonicated for 2 h to reach a homogeneous dispersion.

Supporting electrolytes used for electrochemical experiments were sodium phosphate buffer, 0.1 mol L^{-1} Na_2HPO_4/NaH_2PO_4 (PB), pH 5.5, and 0.1 mol L^{-1} KCl.

Apparatus

Atomic force microscopy (AFM) images of a highly oriented pyrolytic graphite (HOPG; NT-MTD, Ireland) surface bare or covered with GO dispersed in the chitosan solution were recorded with an atomic force microscope NanoWizard 3 AFM (JPK Instruments, Germany) in a dynamic AC mode using Arrow-NCR probes (Nanoworld, Switzerland).

All electrochemical measurements were carried out with a potentiostat/galvanostat CompactStat (Ivium Technologies, The Netherlands) assembled with a three-electrode system. Electrode characterisation was carried out using cyclic voltammetry (CV) and electrochemical impedance spectroscopy (EIS). The working electrode was a bare glassy carbon electrode (GCE, diameter of 3.0 mm) and/or modified GCE, a platinum wire served as the counter electrode and an Ag/AgCl (sat. KCl) electrode was as a reference. EIS was performed with the same equipment at a constant applied potential in a frequency range from 100 kHz to 0.1 Hz, with potential perturbation of 10 mV.

Fixed potential amperometry (FPA) measurements were also performed with the same equipment at the constant applied potential of 350 mV in a continuously stirred (100 rpm) 0.1 mol L^{-1} PB solution. The quantitative amperometric AA determination was carried out using the standard addition method, if not specified otherwise.

Chemical functionalisation of graphene

Graphene was functionalised using the following protocol: 50 mg of graphene was sonicated for 20 h in a mixture of 5 mol L^{-1} H_2SO_4 and 5 mol L^{-1} HNO_3, volume ratio 3:1, at 40 °C; then the mixture was filtrated and neutralised with MiliQ water by continuous washing. Finally, the solid precipitate was dried in an oven at 80 °C for 24 h. After the functionalisation oxy- and carboxy-species are attached to the side or other defect places so this structure is closer to graphene oxide than graphene, therefore further it is indicated as GO.

Preparation of graphene modified glassy carbon electrode

Prior to modification, the GCE surface was carefully polished to a mirror-like plane with 1.0, 0.3 and 0.05 μm aluminium-oxide slurry successively and rinsed with MiliQ-water followed by sonication in water for 1 min after each polishing step. Then the electrode was polished electrochemically in 0.1 M KCl by repeat scanning the applied potential between –1.0 V and +1.0 V versus Ag/AgCl at a scan rate of 100 mV s^{-1} for at least 10 cycles until constant voltammograms (CVs) were obtained. Finally, the electrode was successively rinsed with MiliQ water and left to dry in air.

GCE modified with graphene (G/GCE), graphene oxide (GO/GCE) as well as graphene and graphene oxide dispersed in an aqueous chitosan solution (G-Chit/GCE and G_m-Chit/GCE, respectively) was fabricated applying a similar procedure as reported elsewhere [28–30]. In order to prepare the G/GCE, GO/GCE, G-Chit/GCE and G_m-Chit/GCE, the aliquot of 3 μL of aqueous graphene suspension, immediately after sonication of the suspension for 30 min, was drop-coated uniformly onto the GCE surface and left to dry in air. G_m-Chit/GCE had different GO loads indicated as G_{m1}-Chit/GCE, G_{m2}-Chit/GCE, G_{m3}-Chit/GCE and G_{m4}-Chit/GCE containing 1.5 mg mL^{-1}, 10 μg mL^{-1}, 10 ng mL^{-1} and 10 pg mL^{-1} GO in the suspension, respectively.

In the case of Chit/GCE without graphene, the aliquot of 3 μL of the aqueous chitosan solution immediately after sonication for 30 min (in order to mimic the same conditions) was drop-coated uniformly onto the GCE surface and left to dry in air.

Sample preparation

Pharmaceutical vitamin C powder (100% of ascorbic acid), 0.176 g, was dissolved in 10 mL of water and applied immediately to ascorbate analysis using the calibration method employing CV.

The commercial orange juice sample was prepared as follows: juice was filtered using a high quality filter paper (KA2, diameter of 90 mm, Czech Republic) into dark glass laboratory dishes to prevent ascorbic acid oxidation; a clear filtrate obtained was immediately used for further analysis,

injecting 0.30 mL of the sample to 4.70 mL of 0.1 mol L^{-1} PB solution pH 5.5. AA was determined by FPA using the standard addition method adding aliquots of 100 µmol L^{-1} AA under constant stirring of the working solution.

Green ice tea was used untreated due to its initial transparency and clearness. An aliquot of 0.30 mL of the ice tea sample was added to 4.70 mL of 0.1 mol L^{-1} PB solution pH 5.5. AA was determined like in the case of commercial orange juice.

RESULTS AND DISCUSSION

Electrochemical characterisation

Bare GCE and modified with graphene, chitosan, G-Chit and GO-Chit, immobilised by drop-coating of the corresponding aqueous suspension, were characterised by CV in the presence of the redox compound $K_4Fe(CN)_6$. The results obtained from differently modified electrodes were compared in order to evaluate the quality of immobilisation of graphene and GO which could be further used for sensing applications.

CV was performed in order to study the electrochemical behaviour at the graphene (2.5 mg mL^{-1}) modified glassy carbon electrode. CVs recorded at different potential scan rates ranging from 5 to 300 mV s^{-1} at the bare and graphene modified GCE in 0.1 mol L^{-1} KCl containing 5 mmol L^{-1} $Fe(CN)_6^{4-}$ are shown in Fig. 1. The peaks are well defined for G/GCE and have a slightly broadened wave shape in the case of bare GCE. As seen, G/GCE improved an electrochemical behaviour of the modified electrode. Both cathodic and anodic peak currents increased: Conducting properties of graphene were contributing to the improvement of the electrochemical process at the modified GCE. Data in Table 1 (upper part) showed the dependence of an electroactive surface area,

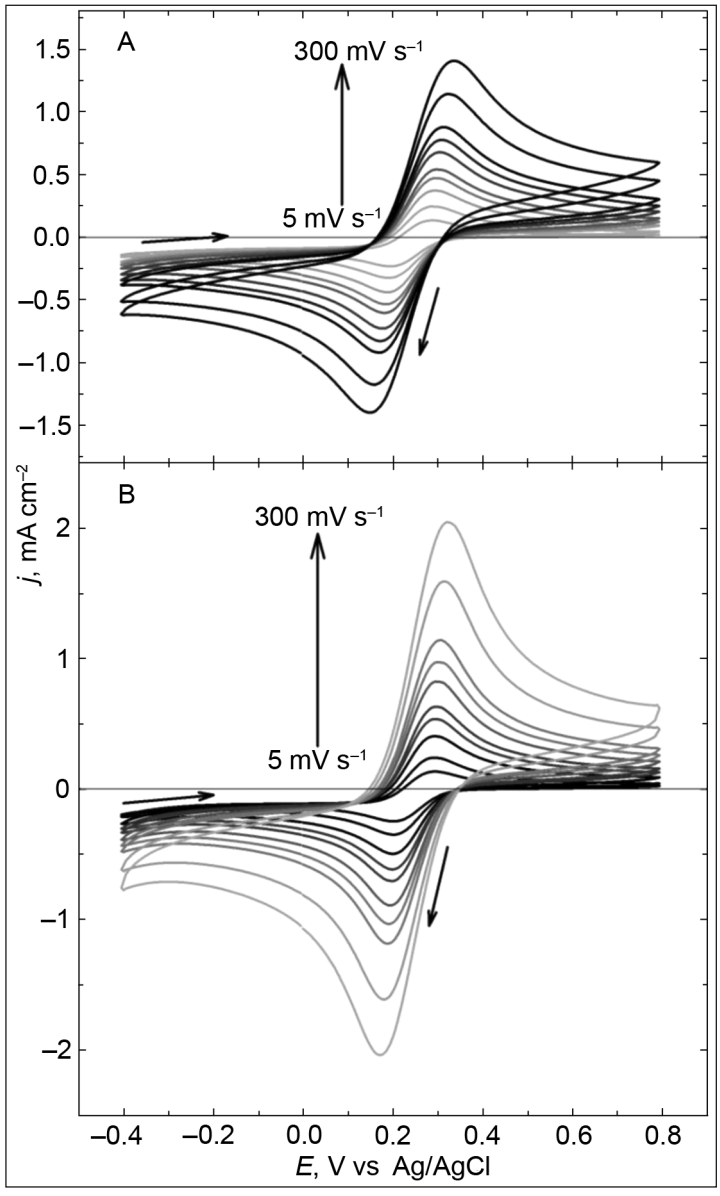

Fig. 1. CVs in 5 mmol L^{-1} $K_4Fe(CN)_6$ and 0.1 mol L^{-1} KCl at different scan rates (in mV s^{-1}): 5; 10; 20; 30; 40; 60; 80; 100; 200; and 300, at A, GCE; B, G/GCE, where graphene load is 2.5 mg mL^{-1}

Table 1. **Electroactive area of glassy carbon electrodes modified with various graphene loads. Calculated from Figs. 1 (GCE1) and 2B (GCE2)**

Electrode	Graphene load, µg mL^{-1}	Electroactive surface area, cm^2
GCE1	–	0.0430
G/GCE1	10	0.0464
	100	0.0545
	1 000	0.0606
	2 500	0.0757
	5 000	0.0771
	7 500	0.0568
	10 000	0.0486
GCE2	–	0.0568
Chit/GCE2	–	0.0436
G-Chit/GCE2	1 000	0.1560
G_{m1}-Chit/GCE2	1 500	0.1884
G_{m4}-Chit/GCE2	0.00001	0.0800

calculated from the Randles-Ševčik equation, on increase in graphene load. Initially, the electroactive area was increasing with an amount of adsorbed graphene but after the concentration reached 5 mg mL^{-1} the area decreased due to week adsorption on the surface. This fact indicated that higher load of graphene is not well attached on the electrode surface and it was washed out even during the first experiment.

In order to resolve this problem, chitosan was chosen to immobilise graphene [21, 31]. CVs at G-Chit/GCE and G_m-Chit/GCE are shown in Fig. 2A; additionally, CVs were also recorded at bare GCE and Chit/GCE in order to study the influence of chitosan. As clearly seen, the response to $Fe(CN)_6^{4-}$ was almost the same at bare and at Chit/GCE electrodes indicating that chitosan has no influence on the electrochemical behaviour but slightly decreased the electroactive surface area of the electrode (Table 1). However, when the electrode was modified with graphene in the aqueous chitosan solution a large increase in the current of the redox peaks occurred. The highest peak was observed for the G_m-Chit/GCE electrode indicating that chemically functionalised graphene was better immobilised in the film.

In all cases, at bare and modified GCE, the dependence of current peak on the square root of the potential scan rate is linear for both oxidation and reduction peaks, which shows a diffusion-controlled process (Fig. 2B). The electroactive surface area was calculated applying the Randles-Ševčik equation and using a diffusion coefficient for $Fe(CN)_6^{4-}$ of $7.6 \cdot 10^{-6}$ cm^2 s^{-1} [32] is presented in Table 1. As seen, the electroactive surface of the G_{m1}-Chit/GCE2 was greater in a factor of 3.3 than that of the bare GCE2.

Figure 3 shows an AFM image of an unmodified HOPG surface (A) and HOPG modified with G_{m4}-Chit (B), prepared

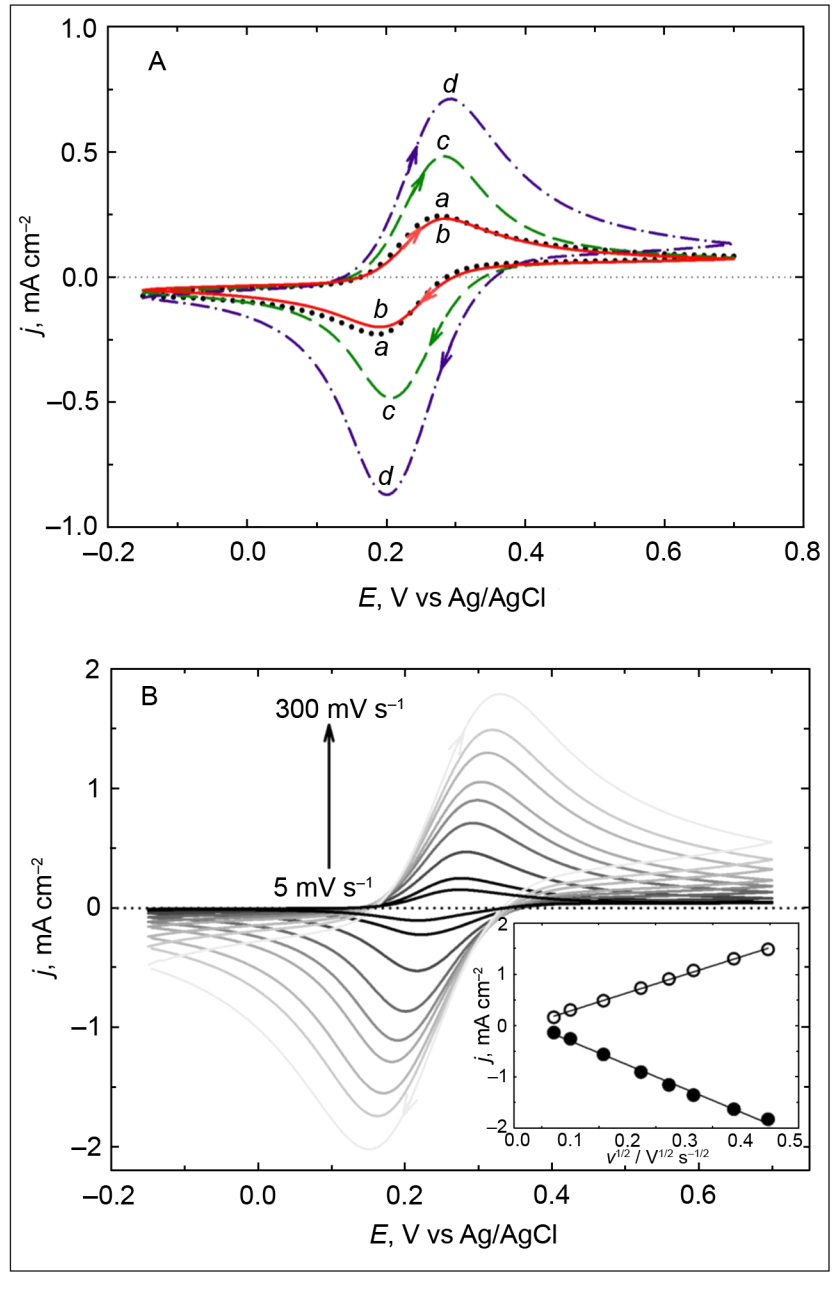

Fig. 2. CVs at A – differently modified electrodes: *a*, bare GCE; *b*, Chit/GCE; *c*, G-Chit/GCE; d, G_m-Chit/GCE, potential scan rate 50 mV s^{-1}; B – G_m-Chit/GCE at different scan rates (in mV s^{-1}): 5; 10; 25; 50; 75; 100; 150; 200; and 300 in 2 mmol L^{-1} $K_4Fe(CN)_6$ and 0.1 mol L^{-1} KCl solution. Inset B – dependence of the peak current on the square root of the scan rate: ● – oxidation; ○ – reduction

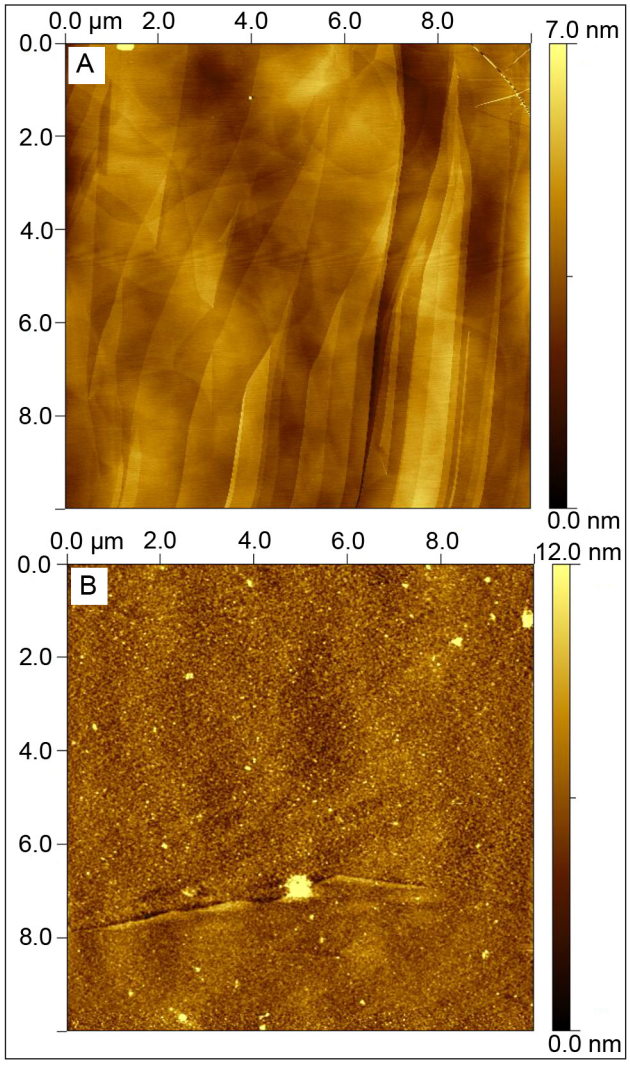

Fig. 3. AFM images of (A) bare HOPG and (B) G_{m4}-Chit/HOPG at open circuit potential in 0.1 mol L^{-1} PB solution, pH 5.5

under the same conditions as G_{m4}-Chit modified GCE. The net of polymer film was clearly visible and no graphene sheets were found credibly being entrapped in the chitosan network since the thickness of the chitosan film was ca. 20–25 nm but graphene sheets had thickness of 2–4 nm as determined previously [15].

Electrochemical application of graphene modified electrode

Cyclic voltammetry

CV in the presence of ascorbate was performed in order to compare an influence of different film modified electrodes on ascorbate oxidation. CVs of 200 μmol L^{-1} AA at bare, G_{m1}-Chit/GCE and G_{m4}-Chit/GCE are seen in Fig. 4. All electrodes exhibited electrochemical response to ascorbate. In the case of bare GCE and G_{m1}-Chit/GCE the oxidation of ascorbate resulted in a broadened peak with the peak potential of 0.36 V and 0.38 V, respectively. Such a modified GCE exhibited a quite low oxidation peak in comparison with low concentration, i. e. G_{m4}-Chit/GCE (peak potential 0.34 V). Larger peak current and better defined peak shape of G_{m4}-Chit/GCE indicated that a lower concentration of GO promoted better electrochemical performance of the electrode than that of the thick graphene load. G-Chit/GCE showed electrochemical response to ascorbate almost equal to that of G_{m1}-Chit/GCE and Chit/GCE similar to bare GCE (not shown).

Furthermore, the calibration of the electrode was performed by CV and the peak current was plotted versus ascorbate concentration in the whole concentration range studied (up to 3 mmol L^{-1}). The parameters characterising electrode / sensor peculiarities were calculated from the calibration curves obtained and presented in Table 2. Linear dependence of the peak current on ascorbate concentration

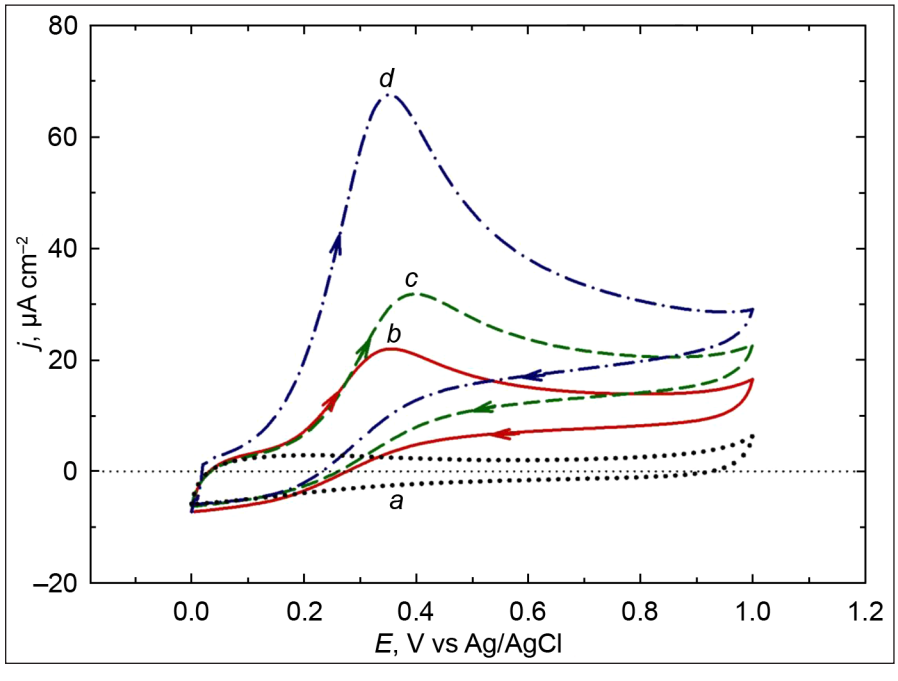

Fig. 4. CVs at *a*, *b*, bare GCE; *c*, G_{m1}-Chit/GCE; *d*, G_{m4}-Chit/GCE in *a*, 0.1 mol L^{-1} PB solution, pH 5.5; *b–d*, presence of 200 μmol L^{-1} of ascorbate. Potential scan rate: 100 mV s^{-1}

Table 2. **Analytical parameters for ascorbate calculated from calibrations curves obtained at various graphene loads modified electrodes using CV in 0.1 mol L^{-1} PB, pH 5.5**

Electrode	Linear range, mmol L^{-1}	Sensitivity, μA mM^{-1} cm^{-2}	LOD, nmol L^{-1}	Correlation coefficient, R^2
GCE	0.1–3.0	56.9 ± 3.1	135	0.990
Chit/GCE	0.01–3.00	96.4 ± 4.2	65.9	0.998
G-Chit/GCE	0.02–3.00	108 ± 5	18.8	0.998
G_{m1}-Chit/GCE	0.01–3.00	98.5 ± 4.1	40.0	0.997
G_{m4}-Chit/GCE	0.02–3.00	247 ± 8	13.3	0.999

showed the ability of the sensor to operate in the whole concentration range studied.

Comparison of the electroanalytical parameters at different electrodes showed that G_{m4}-Chit/GCE possessed 4 times higher sensitivity and 10 times lower limit of detection (LOD) than that of bare GCE (Table 2), indicating faster electron transfer kinetics at the modified electrode. The thick G_m load decreased LOD three times in comparison with the bare GCE. This was due to the different graphene flake arrangement in the thick film [15]; more effective distribution of graphene for direct electron transfer was at its low concentration with no aggregates formed on the electrode surface.

Fixed potential amperometry

Besides CV, FPA was performed to study the sensor response to ascorbate since it is usually a more sensitive analytical method than CV [33], and often more convenient for natural samples. The results obtained from CV data showed that functionalised graphene was more sensitive than unfunctionalised, therefore, the first one was applied for the FPA investigation. Three different electrode modifications were used: G_{m2}-Chit/GCE, G_{m3}-Chit/GCE and G_{m4}-Chit/GCE. The working potential was determined experimentally by scanning potential in the interval from 0.1 to 0.4 V with a step of 50 mV in 0.1 mol L^{-1} PB in the absence and presence of 500 μmol L^{-1} of ascorbate. The graph plotted from the data obtained, potential vs. current (not shown), revealed that the increase in current slows down at 350 mV which was chosen as an optimal working potential. A response time to ascorbate, τ_{95}, at the electrodes used was approximately 3 s at various ascorbate concentrations (Fig. 5). Furthermore, the current increased linearly with increase in ascorbate concentration up to 0.6 mmol L^{-1} (Inset of Fig. 5), and the sensitivity of the electrodes to ascorbate was expressed by the following sequence: G_{m2}-Chit/GCE < G_{m3}-Chit/GCE < G_{m4}-Chit/GCE, i. e. 89 ± 5, 153 ± 6 and 175 ± 6 μA cm^{-2} mM^{-1}, respectively. LOD at these electrodes can also be described by the same sequence being the lowest at G_{m4}-Chit/GCE (20 nmol L^{-1}) and the highest at G_{m2}-Chit/GCE (0.29 μmol L^{-1}). The correlation coefficients were as follows: 0.997 using G_{m2}-Chit/GCE and G_{m3}-Chit/GCE electrodes, and 0.998 when G_{m4}-Chit/GCE was used.

On-line FPA-AFM was performed in the presence of 1 mmol L^{-1} ascorbate at 0.35 V, which was an optimal potential for ascorbate oxidation at this electrode modification. Oxidation current density increased significantly but just a

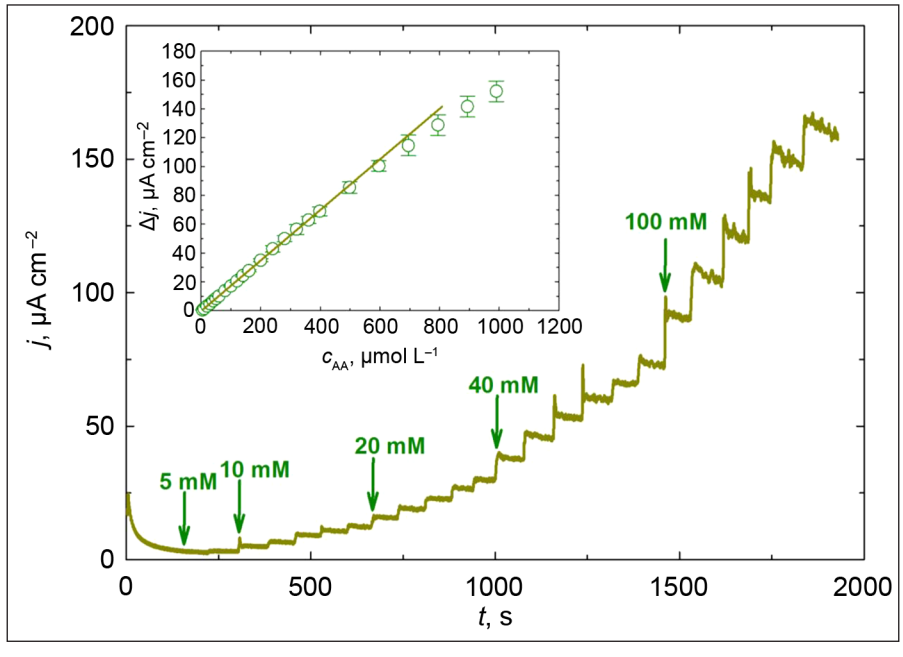

Fig. 5. Amperogram at G_{m4}-Chit/GCE with various additions of ascorbate to 0.1 mol L^{-1} PB solution, pH 5.5. Inset indicates the calibration curve calculated from 3 amperograms under the same conditions

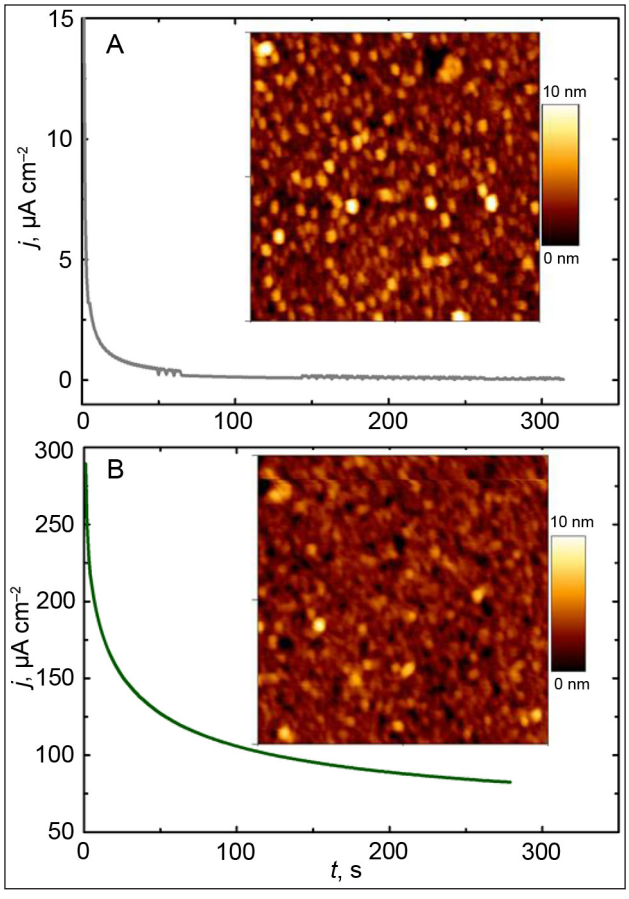

Fig. 6. Current-time curves at G_{m1}-Chit/HOPG modified electrode in 0.1 M PBS at 0.35 V in A, absence, B, presence of 1 mmol L^{-1} ascorbate vs Ag/AgCl(3 M KCl). Insets – AFM images were taken while current-time curves were registered. The scanned area is 1 μm × 1 μm

little change has been observed in topography of the film, i. e. flattening of the polymer net at the surface after ascorbate addition; this indicated adsorption of ascorbate on the electrode surface, as seen in Fig. 6.

Comparison of characteristics of the most sensitive electrode modification, G_{m4}-Chit/GCE, with other modified electrodes reported in literature is presented in Table 3. As seen, ascorbate has been determined at differently prepared sensors and under different operating conditions, applying various electrochemical methods. Most differential pulse voltammetry (DPV) was used in the presence of ureate and / or dopamine. This sensor is not the most sensitive ascorbate detector from the reported in the literature, but the LOD is the lowest in both CV and FPA. Surprisingly, sensitivity was higher using CV for ascorbate determination at this sensor. Moreover, the most sensitive sensors reported in Table 3 had either rather high operating potential, e. g. FPA at 0.57 V vs SCE [40], or electrode preparation was rather long and complicated because redox mediators were used [42, 43]. This sensor was simple to prepare and was most sensitive from similar simple ascorbate sensors; moreover, LOD is the lowest from others reported.

Electrochemical impedance spectroscopy

EIS investigation was performed in order to shed light on ascorbate influence on performance of graphene-modified electrodes. Prior to studies in the presence of ascorbate, electrode was characterised at different potentials in a blank buffer solution (Fig. 7A), and then 0.5 mmol L^{-1} of ascorbate was added for further studies. As seen in Fig. 7A, the shape of the complex plane spectra depends on the processes

Table 3. **Comparison of analytical parameters for AA at different sensors under various conditions. Literature overview**

Electrode	Method	Medium	Linear range, mmol L^{-1}	LOD, μmol L^{-1}	Sensitivity, μA cm^{-2} mM^{-1}	Ref.
G-Chit/GCE	DPV	0.1 M PB (pH 4.0)	0.001–1.678	0.01	No data	[34]
G-Chit/GCE	DPV	0.05 M PB (pH 7.0)	0.050–1.2	50.0	0.12	[35]
MWCNT[a]-PEDOT[b]/GCE	DPV	0.1 M PB (pH 5.0)	0.1–2.0	100	2.23	[36]
SGNF[c]-IL[d]-Chit/GCE	DPV	0.1 M PB (pH 6.0)	0.03–0.35	14.8	1.06	[37]
HCNT[e]/GCE	DPV	0.1 M PB (pH 6.8)	0.008–0.180	0.90	0.005	[26]
PSA[f]/GCE	DPV	0.05 M PB (pH 7)	0.002–1.900	1.70	127	[38]
CPB[g]-Chit/GCE	DPV	0.1 M PB (pH 4.7)	0.02–2.00	No data	2.21	[39]
PVP[h]-Mo$(CN)_8^{4-}$/GCE	FPA, 0.57 V (SCE)	0.1 M H_2SO_4 (pH 0.7)	0.011–0.980	5.50	687	[40]
Cu$(FeCN)_6$/CFE[i]	FPA, 0.05 V (SCE)	0.1 M PBS (pH 7.0)	0.011–5.000	1.30	11.0	[41]
PVC/TTF-TCNQ[j]	FPA, 0.4 V (Ag/AgCl) CV	0.1 M PBS (pH 7.0)	0.077–11.00 0.11–5.00	44.0 1.00	450 110	[42]
MWCNT/PNB[k]/GCE	FPA, 0.0 V (Ag/AgCl) CV	0.1 M PB (pH 5.3)	0.01–0.14 0.05–1.00	2.40 10.1	860 460	[43]
G_{m4}-Chit/GCE	FPA, 0.35 V (Ag/AgCl) CV	0.1 M PB (pH 5.5)	0.005–0.600 0.01–3.00	0.02 0.01	175 247	This work

[a] Multi walled carbon nanotubes;
[b] Poly(3,4-ethylenedioxythiophene);
[c] Stacked graphene platelet nanofibers;
[d] Ionic liquid;
[e] Helical carbon nanotubes;
[f] Poly(sulponazo III);
[g] Cetylpyridine bromide;
[h] Poly(4-vinylpyridine);
[i] Carbon fibre electrode;
[j] Tetrathiafulvalene-tetracyanoquinodimethane;
[k] Poly(Nile blue).

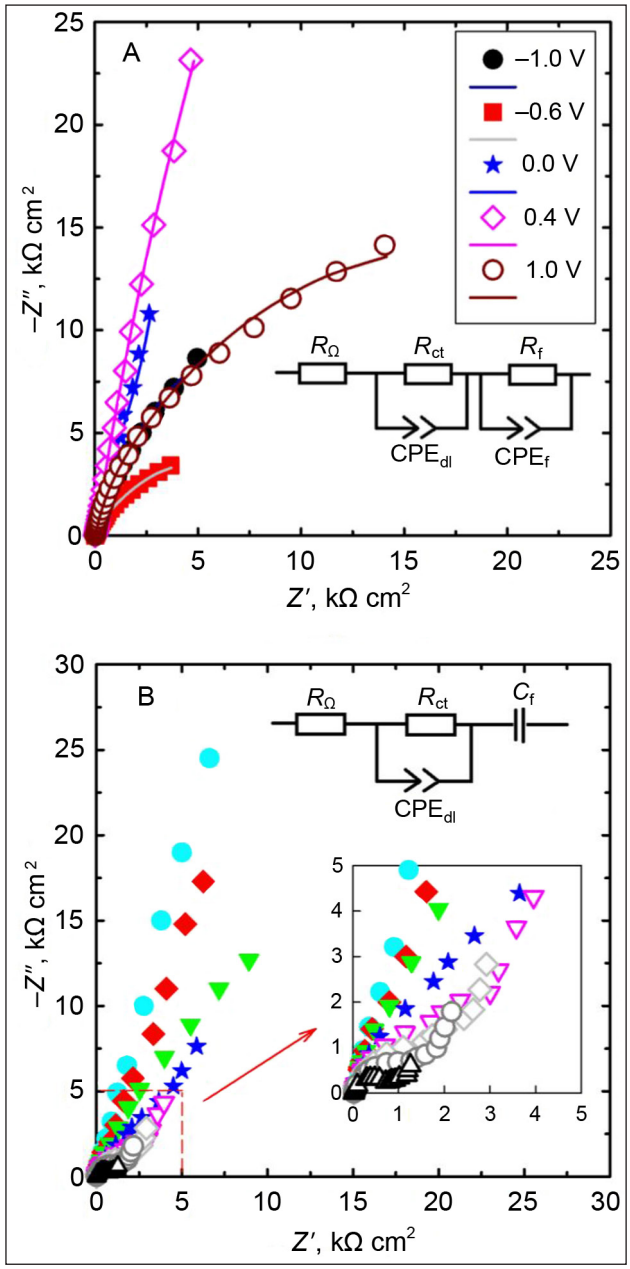

Fig. 7. Electrochemical impedance spectra at G_{m4}-Chit/GCE electrode: A – at different potentials; B – at 0.36 V in the presence of different ascorbate concentrations (in mM): 0 (●); 0.1 (♦); 0.2 (▼); 0.3 (★); 0.5 (▽); 0.7 (◇), 1.0 (○); 2.0 (△). Supporting electrolyte 0.1 M PB solution, pH 5.5. Insets – equivalent circuits used to analyse EIS spectra; inset B – zoom of the indicated area in the square

occurring at that potential: 0.0 and 0.4 V are more capacitive behaviours in the double-layer region; 1.0 V is oxidation of carbon based materials and the graphene-modified surface and, therefore, a shape of spectrum changes; –0.6 V is attributed to reduction of surface oxy-species at carbon electrode; at –1.0 V hydrogen evolution occurs.

The complex plane spectra were analysed applying fitting to electrical equivalent circuits. Most of the spectra (from –1.0 V to –0.4 V and 1.0 V) were fitted to the equivalent circuit indicated in the inset of Fig. 7A, comprising cell resistance, R_Ω, in series with two parallel couples R-CPE, where CPE is a constant phase element as a non-ideal capacitor:

$$CPE = -1/(Ci\omega)^n,$$

where C is the capacitance and it describes the charge separation at the double-layer interface, ω is the angular frequency, and the n exponent is due to the heterogeneity of the surface. The first couple contains the charge transfer resistance, R_{ct}, and non-ideal double-layer capacitor, CPE_{dl}, whilst the second parallel couple consists of the film resistance, R_f, and non-ideal film capacitor, CPE_f. The other spectra were fitted with the film capacitance, C_f, instead of the second R-CPE couple (inset of Fig. 7B). The data of the spectra analysis at high and middle frequency region are presented in Table 4, where the charge transfer was observed. As seen, R_{ct} and double-layer capacitance were slightly decreasing with increase in the potential from –1.0 V to 0.0 V showing that the easiest charge transfer is close to 0.0 V, where no electrochemical process occurred. The highest R_{ct} in this potential region is at –1.0 V due to slight hydrogen evolution. When the potential increased and shifted towards positive values, R_{ct} suddenly rose significantly at 0.2 V and continued to rise until 0.4 V and then slightly dropped at 1.0 V. The charge transfer in this potential region was more difficult due to changes of the chitosan film structure while the electrode was polarized positively.

Addition of AA to the buffer solution significantly changed the R_{ct}. The spectra were recorded only in the positive potential region where oxidation of AA usually occurred. At 0.0 V, where ascorbate was not yet oxidised at G_m-Chit/GCE, R_{ct} increased more than 10 times comparing with the spectrum without ascorbate (Table 4) confirming that no oxidation took place at this potential. An opposite effect is observed at 0.2 V, where R_{ct} decreased more than 10 times after ascorbate addition to the buffer solution. This revealed that AA oxidation was very fast at this potential. Significantly smaller decrease in R_{ct} was observed at 0.4 V, where, according to CV, ascorbate is oxidised. According to impedance data, the fastest ascorbate oxidation occurred at 0.2 V.

Further on, the influence of AA concentration to the electrochemical process was also studied by EIS. The results are presented in Fig. 7B. As seen, no influence was observed down to 0.1 mmol L^{-1} ascorbate. The spectra were recorded at the peak potential, 0.36 V. However, with further increase in ascorbate concentration a capacitive shape of complex plane plots was changing to a semicircle part and a linear part at low frequencies; at the same time impedance values were decreasing indicating a faster electrochemical process. Figure 8 shows changes of R_{ct} and CPE_{dl} with increase in ascorbate concentration. A fast decrease in charge transfer resistance is observed up to 0.3 mmol L^{-1} ascorbate and then the decrease is very slow. Comparison of the double-layer capacitance over the whole range of ascorbate concentrations studied showed a slight increase in the C_{dl} observed up to

Table 4. **Impedance parameters at G_{m4}-Chit/GCE at different applied potentials in 0.1 mol L^{-1} phosphate buffer, pH 5.5, in the absence and presence of ascorbate. The data are calculated from the EIS spectra in Fig. 7A**

E, V	c_{AA}, mmol L^{-1}	R_{ct}, kΩ cm^2	C_{dl}, μF cm^2 s^{n-1}	*n*
–1.0	–	24.5	70.8	0.940
–0.6	–	7.11	81.6	0.933
–0.4	–	6.31	83.8	0.886
–0.2	–	7.58	85.3	0.924
0.0	–	3.73	86.8	0.899
0.0	0.5	44.6	25.2	0.888
0.2	–	30.8	11.6	0.810
0.2	0.5	3.69	9.33	0.834
0.4	–	58.5	51.8	0.878
0.4	0.5	30.3	83.5	0.900
1.0	–	37.6	37.4	0.946

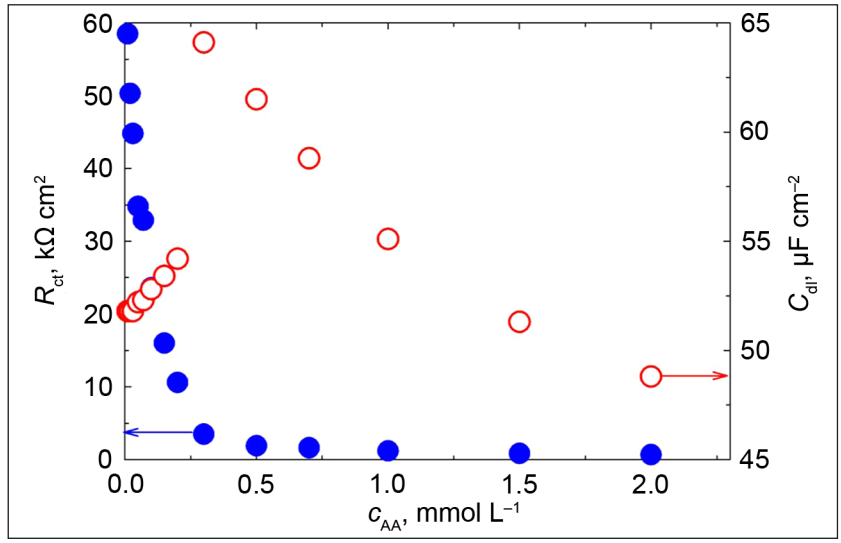

Fig. 8. R_{ct} and C_{dl} changes with increase in ascorbate concentration calculated from the electrochemical impedance spectra in Fig. 7B

0.2 mmol L^{-1} of ascorbate, and then a sudden increase from 28 to 58 μF cm^{-2} was visible in the concentration range from 0.2 to 0.3 mmol L^{-1} of ascorbate. With the further increase in concentration, the C_{dl} decreased linearly up to 1.0 mmol L^{-1}. This plot revealed that control of ascorbate electrooxidation was different at concentrations up to 0.3 mmol L^{-1}, i. e. at low concentrations diffusion controlled the electrochemical process. Meanwhile, at higher concentrations, ascorbate adsorption at the electrode surface had much larger influence and, therefore, the decrease in the C_{dl} was observed and the diminishing of the charge transfer turned to much slower.

Reproducibility, stability and interference

Reproducibility of the sensor G_{m4}-Chit/GCE was 95% (n = 5). Repeatability of the response was related to the stability of the sensor: Long term stability of the sensor, when electrode performance was tested once per day, was not less than 15 days with a signal drop in 5%. On-shelf-stability was at least 40 days.

Interference studies were carried out employing FPA at the same G_{m4}-Chit/GCE. Compounds usually present in fruits were used as interferents, such as glucose, fructose, citric acid and hydrogen peroxide 1, 10 and 20 mmol L^{-1} of each, these compounds were added to 1 mmol L^{-1} of ascorbate in 0.1 mol L^{-1} PB, pH 5.5. Only 20 mmol L^{-1} of citric acid caused a decrease in response current in 1.2% of the original response of ascorbate (not shown).

The obtained results showed that G_{m4}-Chit/GCE can be successfully used as a reproducible, sensitive and stable sensor for ascorbate, which can be determined with this sensor without interference in fruits and juices.

Ascorbate determination in natural samples

In order to evaluate the applicability of the proposed sensor to the determination of AA, it was tested in some real and natural samples. First, the sensor was used for a pharmaceutical vitamin C powder in order to test a reliability of the electrode. Further, soft drinks were used as natural samples to determine AA.

The measurements were carried out in 0.1 mol L^{-1} PB (pH 5.5) at G_{m4}-Chit/GC as in the case of the modelled ascorbate solution; the potential range was from –0.2 to 0.8 V

with intensive stirring after addition of vitamin C sample prior to recording of each CV. The quantitative AA determination was performed using the calibration curve obtained with AA under the same conditions. The results were reliable and showed a true concentration of the prepared vitamin C (not shown). The same results were obtained using FPA.

AA determination using FPA was performed in commercial orange juice (AA concentration given by manufacturer was 30 mg in 100 g or 1.70 mmol L^{-1}) and green ice tea. The current-time response was performed as described in Experimental. The calculated values of AA in the samples determined from 3 measurements are as follows: in commercial orange juice 1.73 ± 0.12 mmol L^{-1} was found and in ice tea 2.35 ± 0.15 mmol L^{-1}. Since the amount of AA in ice tea was not indicated by the producer, the recovery studies have been performed and the recovery of 98% was obtained from 5 measurements with 0.5 mmol L^{-1} AA. As seen, the sensor response was in a good agreement with the provided concentration therefore it can be a reliable sensor for other drink samples.

CONCLUSIONS

The glassy carbon electrode was modified with graphene and GO. The best electrochemical properties were achieved when the electrode surface was modified with a thin film of GO dispersed in an aqueous chitosan solution. The largest electroactive area, calculated from electrochemical behaviour in the presence of $Fe(CN)_6^{3-/4-}$, was obtained at the electrode modified with GO load of 1.5 mg mL^{-1}.

Despite a large electroactive area, the best electrochemical response to ascorbate was exhibited by GCE modified with 10 pg mL^{-1} GO dispersed in the aqueous chitosan solution in comparison with bare, Chit/GCE, G-Chit/GCE and G_{m1}-Chit/GCE electrodes. The sensitivity to the AA of G_{m4}-Chit/GCE electrode was greater in a factor of 4 and the LOD was lower in a factor of 10 in comparison with bare GCE. This electrode can be used as a stable and robust sensor for ascorbate determination. This electrode could be also used as an impedimetric sensor for ascorbate.

AA content was successfully determined at this electrode in natural samples using cyclic voltammetry and fixed potential amperometry. The results were in a good agreement with the concentrations provided by producer.

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ANGLIES ELEKTRODŲ, MODIFIKUOTŲ GRAFENU IR CHITOZANU, TAIKYMAS NUSTATANT ELEKTROCHEMINĮ ASKORBATĄ

S a n t r a u k a

Grafenas (G) ir grafeno oksidas (GO) buvo naudoti stikliškojo anglies elektrodo (GCE) modifikavimui, kurio tikslas – pagreitinti elektronų pernašą. Geriausi rezultatai gauti, kai elektrodas buvo padengtas plona plėvele GO, disperguoto vandeniniame chitozano tirpale. Gauti elektrodai charakterizuoti ciklinės voltamperometrijos (CV) ir elektrocheminės impedanso spektroskopijos (EIS) metodais. Optimizuotas elektrodas buvo panaudotas askorbato analizei modeliniuose ir natūraliuose bandiniuose. Tokio elektrodo jautris askorbatui buvo panašus kaip literatūros šaltiniuose – 247 ± 8 μA cm^{-2} $mmol^{-1}$ L, o nustatymo riba – daug mažesnė nei kitų publikuotų askorbato sensorių – 13 nmol L^{-1}. Duomenys, gauti naudojant šį elektrodą nustatant askorbatą sultyse, yra paklaidų ribose, palyginti su gamintojų pateiktomis askorbato koncentracijomis.