

Screen-printing of electrical sensor for glucose determination with exfoliated graphite-based paste

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Abstract: In this paper, the properties of electrochemical sensor made of screen-printing technology application with graphite-base paste were investigated. The main electrically conductive component in the original paste developed in this study is exfoliated graphite. The electrodes were studied using optical and scanning electron microscopy methods to confirm the quality and integrity of the screen-printing process results. Electrochemical measurements (chronoamperometry and cyclic voltammetry) of printed electrodes show that the performance of this exfoliated graphite-based paste is similar to that of a commercially available graphite paste despite the radically lower content of carbon. The biosensor prototype manufactured in this work is characterized by anisotropic surface morphology formed by mixed carbon black with milled carbon particles (exfoliated graphite and microcrystalline graphite). This prototype has a very good linearity of response to glucose in a wide range from 1 to 40 mM, while manifesting the values of currents and sensitivity comparable to a commercially available analogue.

Keywords: electrochemical measurements; exfoliated graphite; glucose biosensor; graphite electrode; graphite paste; screen-printing.

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Трафаретная печать датчика для измерения глюкозы из пасты на основе терморасширенного графита

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Аннотация: Исследованы свойства электрохимического датчика, изготовленного с помощью технологии трафаретной печати графитовой пасты. Основным электропроводящим компонентом пасты является терморасширенный графит. Для подтверждения качества и целостности результатов трафаретной печати электроды исследованы методами оптической и сканирующей электронной микроскопии. Электрохимические измерения (хроноамперометрия и циклическая вольтамперометрия) печатных электродов показали, что характеристики пасты на основе терморасширенного графита аналогичны характеристикам коммерчески доступной графитовой пасты, несмотря на радикально меньшее содержание углерода. Прототип биосенсора, изготовленный в данной работе, характеризуется анизотропной морфологией поверхности, образованной смесью технического углерода с измельченными частицами углерода (терморасширенный и микрокристаллический графит). Данный прототип обладает хорошей линейностью реакции на глюкозу в широком диапазоне от 1 до 40 мм, проявляя при этом значения токов и чувствительности, сравнимые с коммерчески доступным аналогом.

Ключевые слова: электрохимические измерения; терморасширенный графит; датчик глюкозы; графитовый электрод; графитовая паста; трафаретная печать.

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1. Introduction

Graphite pastes are used for screen-printing and are the main material for the manufacture of electrodes in biosensors [1–4]. The search for inexpensive and reliable solutions to meet the growing demand for electrochemical biosensors with which quantitative measurement of glucose concentration in human blood can be carried out quickly remains relevant. Over the years, a large number of different materials have been tested in graphite paste formulations for screen-printing. However, only a few compositions have been partially disclosed and presented in the public domain.

There are several manufacturers in the research area reporting successful implementation of commercial graphite pastes: Acheson Colloids Company (UK), Gwent Electronic Materials (UK), DuPont (USA), Ercon (USA) and Nippon Graphite Industries Ltd (Japan). As found in the literature, the products of Gwent Electronic Materials demonstrate good reproducibility of the electrical conductivity of the electrodes and the best characteristics of sensitivity to a wide range of detectable substances [5–7], in particular, to glucose [8, 9]. This is often used as a reference in the research related to the creation of the original formulations of graphite paste and printed electrodes based on it.

Most of the recent works are devoted to modification of a commercially available graphite paste by adding various materials to its composition [10–12]. These modifications can change rheological behavior of a graphite paste and improve physicochemical properties of printed electrodes. For example, such modification may increase electrode surface area or sensitivity; it may also influence the kinetics of electronic transport. The most common nonmetallic conductive fillers of conductive pastes are carbon black, glassy carbon, graphite, carbon nanotubes and graphene [13–17].

From this point of view, exfoliated graphite (EG) is a promising material for the manufacture of graphite electrodes obtained by screen-printing [18]. EG is characterized by low electrical resistivity, thermal stability and chemical inertness [19, 20]. However, due to the low density of EG, it is difficult to maintain the high-quality level of printed electrodes. A solution to this problem may be the co-

introduction of additional carbon materials into the EG-based paste, such as microcrystalline graphite (MG) and carbon black (CB) [21].

The purpose of this work is to develop, manufacture and investigate the properties of EG-based printed electrodes for electrochemical biosensors for determining glucose concentration.

2. Materials and Methods

2.1. Source materials

Suspension polyvinyl chloride (PVC) grade C-7059 M (CAUSTIC, Russia), 1,2,3,4-tetrahydronaphthalene (THN) (qualification "Purified", LLC "VitaChem Kazan", Russia), N-methylpyrrolidone (N-MP) (qualification "Purified", ECOS-1, Russia), 3-glycidioxy-propyltrimethoxy-silane (LT560, Jiangxi Chenguang New Materials Co., Ltd., China) were used to prepare the polymer base for exfoliated graphite paste (referred to as EGP below in the text). The main electrically conductive component is EG (UNICHIMTEK, Russia). Additional carbon materials were also used: MG grade "MG-1" (NIIgrafit, Russia) and CB brand "N375" ("YATU named after V.U. Orlov", Russia).

The EGP has been tested in comparison with the commercially available analogue of GWENT C2130814D2 (referred to as CGP below in the text) (Gwent Electronic Materials, UK).

2.2. Production of graphite paste

The use of a screen mesh when printing graphite electrodes imposes a limit on the size of the solid components of the paste. In this work, their size should not exceed 40 μm , so the original carbon materials were ground. The grinding of EG particles was carried out using an Ultrasonic Disperser Vibracell VCX750 (Sonics & Materials, Inc., USA) in acetone medium. A PULVERISETTE 7 premium line planetary ball mill (FRITSCH GmbH, Germany) was used to grind MG. The CB consists of nanoscale particles (~ 50 nm), so it does not require pre-grinding. The finished carbon components were introduced into a liquid polymer base and stirred until a visually homogeneous mass was obtained. The mass content of solid and liquid components of graphite pastes is presented in Table 1.

Table 1. Solid and liquid components of graphite pastes

Component, wt. %	CGP	EGP
Solid components, total	34–38	16
Liquid components, total	62–66	84

Manufacture of graphite electrodes

The screen printing of the studied graphite pastes was carried out on an automatic machine WSC-160 B (WINON Industrial Co., Ltd., China). Sheets of polyethylene terephthalate (PET) were used as a substrate. The heat treatment conditions of graphite electrodes in the drying oven for their fixation on the PET substrate for EGP were 90 °C for 45 min, CGP – 100 °C for 3 h. The conditions are selected so as to minimize the effect of temperature on the original shape of the substrates. Geometric parameters of the finished electrodes: length – 38 mm, width – 1 mm, thickness – 11 µm.

2.3. Preparation and characterization of biosensors

The morphology and surface defects of the graphite electrodes, as well as the size of the conductive particles, were investigated by scanning electron microscopy (SEM) using a TESCAN Vega 3 electron microscope (TESCAN, Czech Republic). Print quality control (geometry of the pattern, through pores, etc.) was carried out by high-resolution optical microscopy using an Olympus BX-51M optical microscope (Olympus Corporation, Japan).

The thickness of printed electrode was evaluated based on the average of five measurements by MITUTOYO digital indicator ID-F125E with stand (MITUTOYO, Japan).

The electrode resistivity was evaluated by means of a four-probe method on a Keithley 2400 Standard Series Source Measuring Unit instrument (Keithley Instruments Inc., USA) at room temperature (~300 K).

Electrochemical measurements were performed according to a three-electrode circuit using printed graphite electrodes. EmStat3 potentiostat (PalmSens BV, Netherlands) was used to register electrode signals. Buffer and aqueous solutions were prepared using pure deionized water purified at Milli-Q (Millipore, USA).

Cyclic voltamperograms (CV) were recorded in a potassium-phosphate buffer solution (pH 7.4) with a potential scanning rate of 40 mV·s⁻¹ in the range from -0.40 to 0.75 V. The printed electrode of the sample under study was used as a working electrode, a silver chlorine electrode was used as a reference electrode (Ag/AgCl), a graphite rod was used as an

auxiliary electrode. All electrodes were immersed in glass containing a working solution. The area of the submerged part of the working electrode was 0.07 cm². To verify the reproducibility, measurements were made on at least two independently printed electrodes.

Chronoamperograms (CA) were recorded at a potential of 0.3 V in a potassium-phosphate buffer solution with the addition of sodium chloride (10 mM KH₂PO₄, 40 mM K₂HPO₄, 184 mM NaCl, pH 7.4) containing glucose at concentrations 0.6 to 50.0 mM. A printed graphite working electrode, an auxiliary electrode and a reference electrode were used to detect currents. The enzyme immobilization was carried out in such a way: an aqueous solution (0.9 µL) of glucose oxidase (10 mg·mL⁻¹) and potassium hexacyanoferrate (III) (113 mg·mL⁻¹) were placed to the working area by means of microdozer and then left to stabilize at a room temperature. The printed electrodes with a substrate were fixed horizontally and a portion of the working electrolyte with a volume of 2 µL was applied to the working area using a microdozer. At least three CA were recorded for each glucose concentration, a new printed electrode was used for each measurement.

3. Results and Discussion

The original EG powder consisted of macroparticles of various shapes and sizes formed from interconnected ~150–250 µm graphite plates. Ultrasonication of the original EG led to their destruction to separate, predominantly flat particles of irregular shape. By the means of SEM method the particle sizes of the EG after the US was estimated (Fig. 1).

According to Fig. 1, based on the established parameters of the screen mesh of 40 µm, 84 % of the EG particles pass threshold. The size of a third of all particles after ultrasonication (33 %) is 20 – 25 µm.

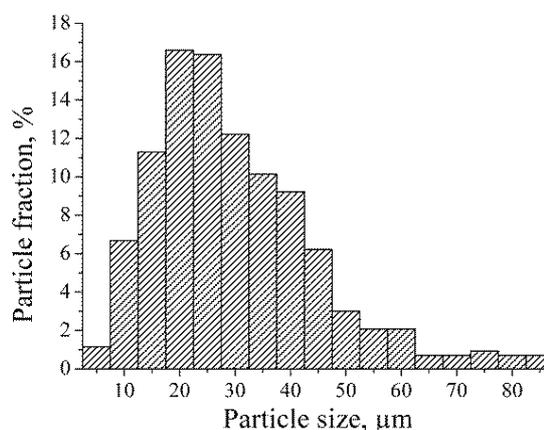


Fig. 1. Distribution histogram of the EG particle size after ultrasonication

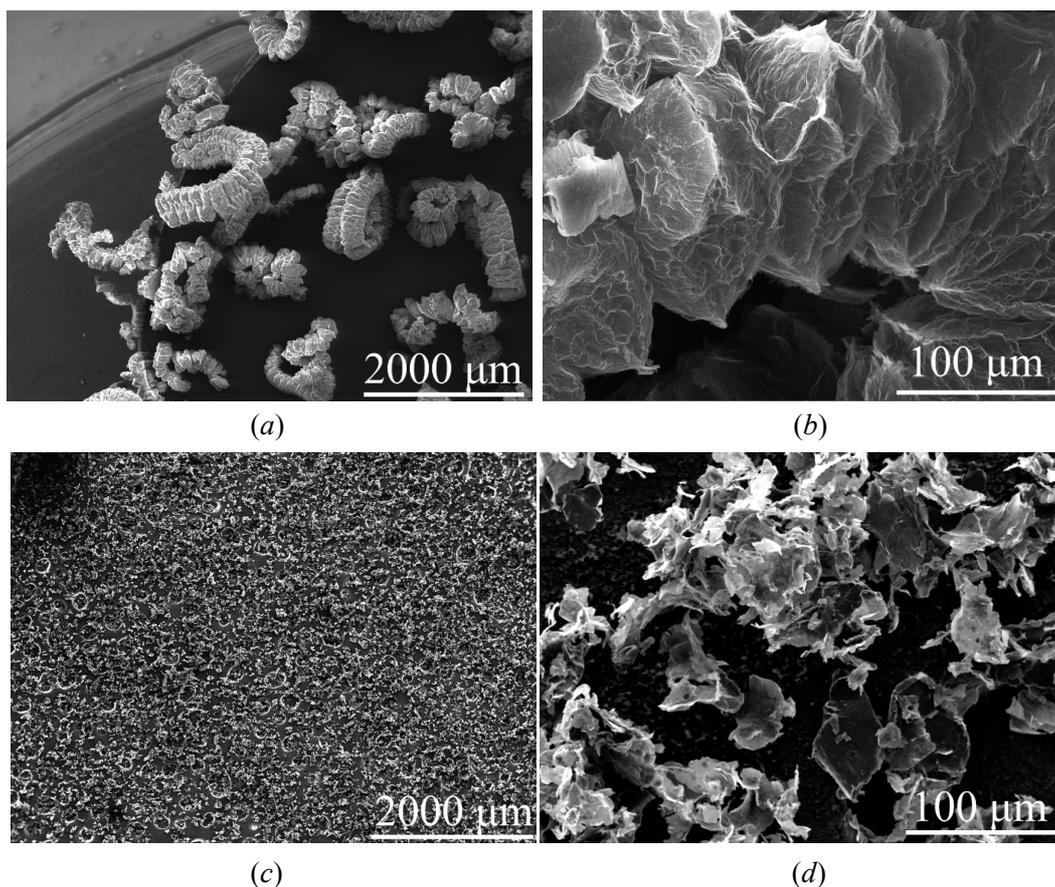


Fig. 2. SEM micrographs of the EG particles: (a, b) initial particles; (c, d) particles after ultrasonication

The result of the ultrasonication of the original EG powder is presented in Fig. 2.

The original MG powder consisted of individual particles ranging in size from 0.1 to 2.0 mm. As a result of grinding in a planetary mill, the size of most MG particles (91 %) did not exceed 40 μm, while particles ranging in size from 5 to 15 μm made up the main share (67 %) (Fig. 3).

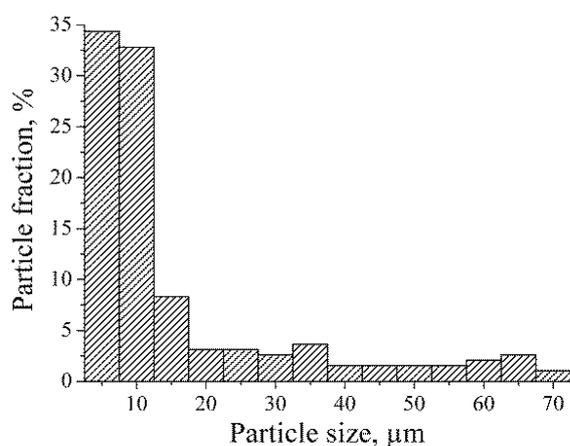


Fig. 3. Distribution histogram of the MG particles size after grinding in a planetary mill

The SEM micrographs for the MG powder before and after grinding in a ball mill are presented in Fig. 4.

The physical properties of the graphite electrode depend on the number of defects that can emerge during the process of screen-printing or be formed due to the incompatibility of the materials that make up the graphite paste with each other. The most common defects of printed electrodes are uneven thickness, unfilled areas due to blockage of mesh cells, lack of reproducibility when measuring electrical resistance, and breakdowns between the working, auxiliary, and reference electrodes due to the spread of the paste immediately during printing.

According to the passport data, CGP graphite paste contains 34–38 wt. % of solid components (Table 1). Fig. 5 shows the results of optical microscopy and SEM of CGP paste-based printed graphite electrode.

As can be seen from the microphotographs, the electrode surface is uniformly smoothed and completely filled with carbon particles (Fig. 5b). There is almost no separation of the polymer base (Fig. 5a) along the entire perimeter of the electrode, and there are no spreads either. The boundaries of the electrode have a clear shape, close to rectangular, but its contour is slightly blurred.

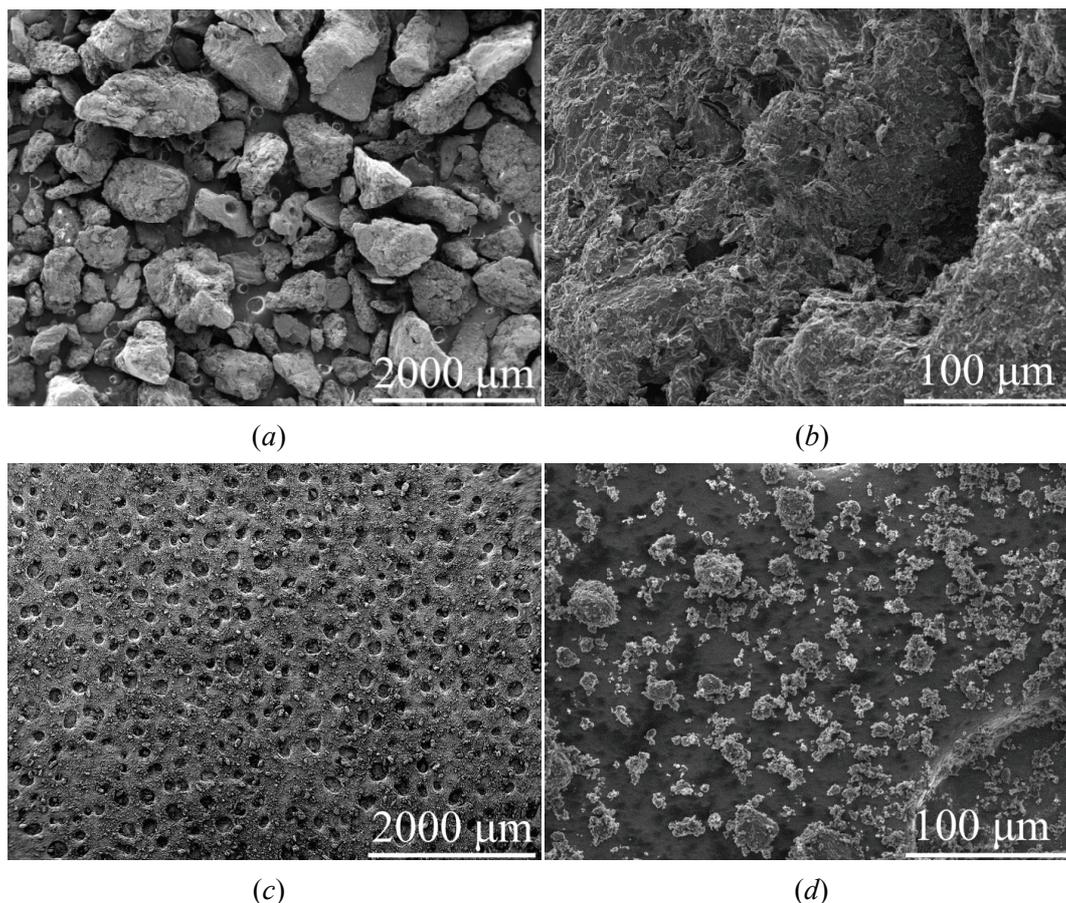


Fig. 4. Scanning electron micrographs of the particles MG: (a, b) original particles; (c, d) particles after ball milling

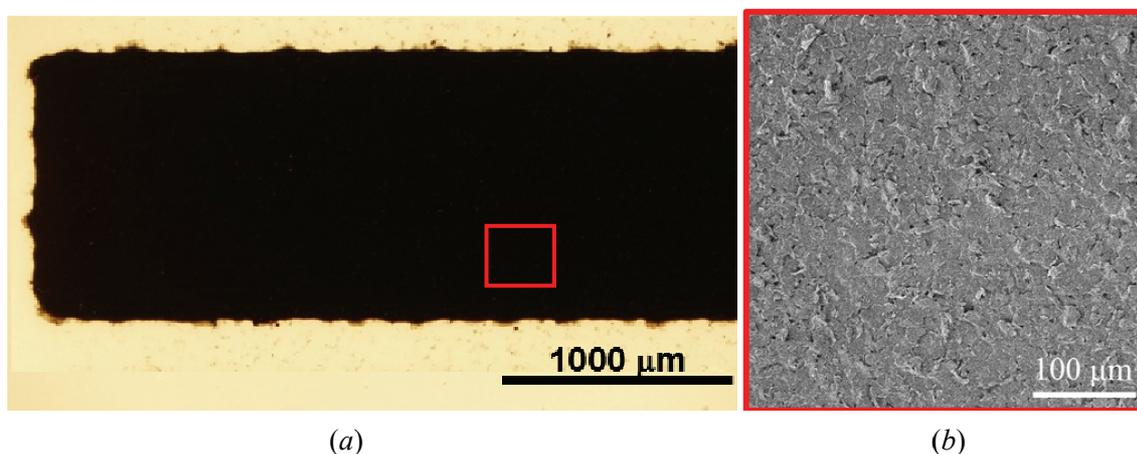


Fig. 5. Images of a printed graphite electrode based on CGP paste:
(a) optical micrograph of the edge; (b) scanning electron micrograph of the surface

Fig. 6a presents a graphite electrode manufactured with the original EGP.

It can be seen from microphotographs that the electrode surface looks loose, but the edges of the EG particles are smoothed by the polymer base, and the filling of the electrode volume with graphite paste components is uniform (Fig. 6b). The separation of the polymer base (Fig. 6c) at the electrode boundaries

is practically absent, there is no spreading, and the electrode border has a wave-like shape.

As a result, the obtained graphite electrodes based on EGP had a rectangular shape with a wave-like contour, without traces of spreading, with a weak separation of the polymer base around the perimeter and uniform filling of the volume with conductive components. The specific electrical resistance values

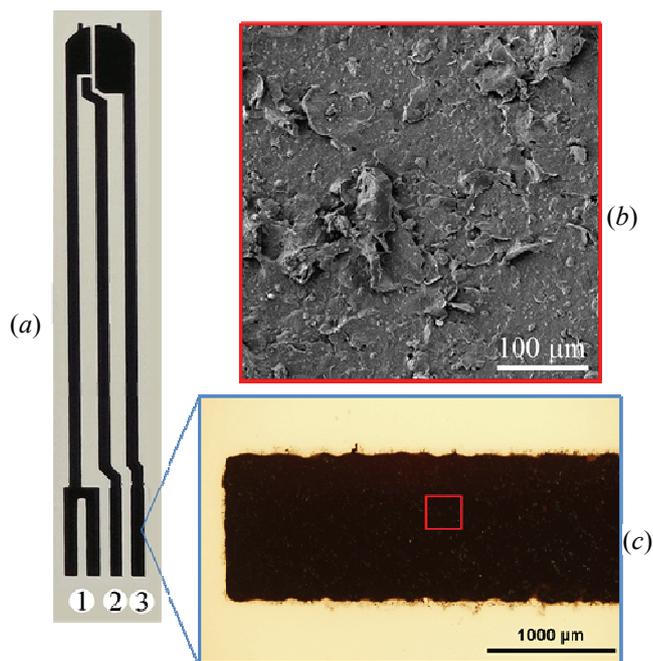


Fig. 6. (a) printed graphite electrode, (1) auxiliary, (2) reference and (3) working electrodes; (b) scanning electron micrograph of the EGP surface; (c) optical micrograph of the EGP edge

of the CGP and EGP are different but similar to each other (Table 2).

Compared to CGP graphite paste, the use of EG as the main electrically conductive component of EGP can reduce the content of solid components by almost two times (see Table 1). It was found that the reduction in the specific electrical resistance of graphite electrodes was influenced by the use of nanoscale CB in the paste [22]. The addition of crushed MG facilitated the passage through the screen mesh of more EG particles up to 40 μm in size (Fig. 7). Corresponding EGP paste volume resistivity value is performing at the same level as EtOH1-5, HMP1-5 and PVP1-5 graphite ink samples with 35 wt. % conductive carbon components, it should be noted that comparable samples may have a lower roughness as in this work it was not investigated [21].

Table 2. Physical characteristics of graphite electrodes

Paste	Electrode thickness, μm	Electrical resistance of the working electrode, Ω	Specific electrical resistance, (Ω×mm ²)·m ⁻¹
CGP	10	1000	270
EGP	11	1500	440

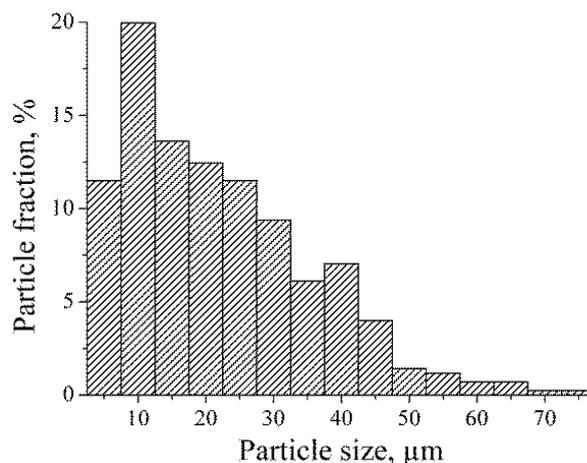


Fig. 7. Histogram of particle size distribution in EGP sample after screen-printing

The chosen ratio between EG, MG and CB in graphite paste led to the formation of an effective electrically conductive network in the printed electrodes.

To compare the electrochemical characteristics of the printed electrodes, voltamperometric and chronoamperometric studies were carried out.

Figure 8 shows the CV of graphite electrodes based on CGP and EGP.

In both cases the CV have a smooth shape without ohmic defects and do not contain extraneous redox peaks. CV are reproduced uniformly for all the printed electrodes. In the case of CGP-based electrodes, the CV is slightly more symmetric and has a slightly wider dual electric layer recharge region than for EGP-based electrodes.

Figures 9 and 10 show the calibration dependencies obtained from the corresponding CA of the samples under study.

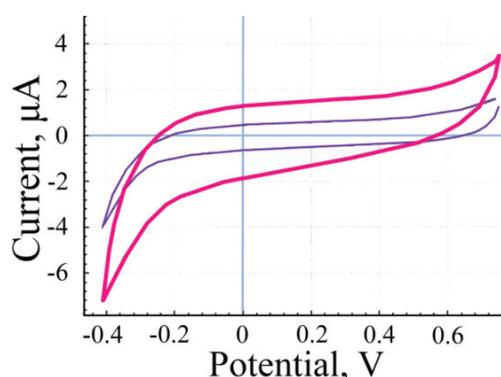


Fig. 8. CV of printed electrodes: graphite electrode based on CGP (purple line); graphite electrode based on EGP (pink line)

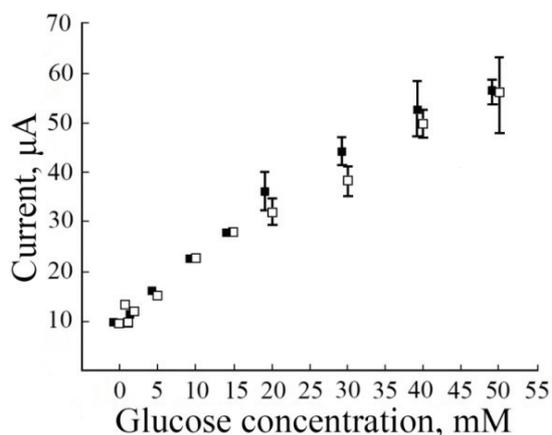


Fig. 9. Calibration dependencies “current – glucose concentration” for graphite electrodes: ■ – CGP; □ – EGP

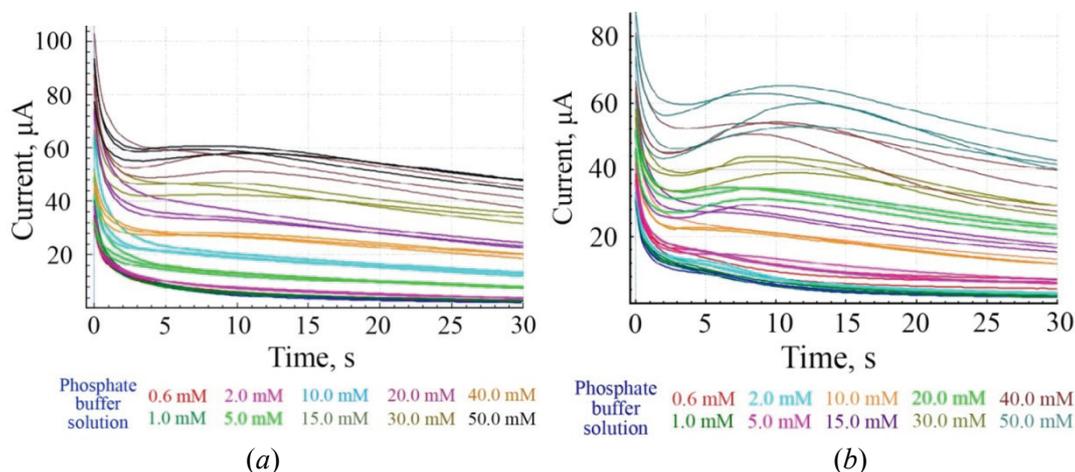


Fig. 10. Chronoamperometry results at different glucose concentrations for graphite electrode based on (a) CGP; (b) EGP

Table 3. Calibration dependency parameters for electrodes based on CGP and EGP pastes

Paste	Sensitivity at 30 mM, $\mu\text{A}/\text{mM}$	Current density at 30 mM, mA/cm^2	Linear range, mM
CGP	1.50	0.7	1–40
EGP	1.30	0.6	

The studied electrodes manifest similar currents and current densities at glucose concentrations of up to 30 mM, as well as the same linear range and similar sensitivity (Table 3). It can be concluded that with the enzymatic oxidation of glucose on printed electrodes based on EGP are not inferior to electrodes obtained on the basis of CGP. It should be noted that such a wide linear range of glucose detection is not presented among chemical sensors screen-printed by nanomaterials based inks, which are better suited for low concentration glucose detection [4].

As it shown in Fig. 10, printed electrodes based on both graphite pastes exhibit a quasi-linear response to glucose under different concentration levels. Each graph point is an average value of current measurements for three same graphite electrodes at corresponding conditions. In case of glucose concentrations up to 20 mM measurement error ranges are too small, which means good measurement reproducibility. The reproducibility of currents on three independently printed electrodes is good at low glucose concentrations (up to 10 mM). At higher concentrations, reproducibility is impaired in the case of both pastes (to a greater extent in the case of EGP-based paste), which may be due to both different contribution of the ohmic potential drop because of local differences in the electrical conductivity of the printed electrodes and to some heterogeneity of the available surface. The calibration dependencies parameters are presented in Table 3.

4. Conclusion

The EG-based graphite paste studied in this research is suitable for creating electrochemical biosensors manufactured by screen-printing technology. The EG addition into the graphite paste allows the content of carbon-conductive particles to be reduced by up to 16 wt. %, maintaining a low sheet resistivity of the resulting electrodes, and thus reducing the cost of its manufacture. Analysis of the electrode surface by optical microscopy and SEM, as

well as the results of electrochemical tests on the enzymatic oxidation of glucose, shows that the EG-based paste with basic properties is not inferior to the commercially available analogues and is suitable for screen-printing of electrodes that work as a biosensor for glucose quantitative determination in a wide linear arrangement with good reproducibility.

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7. Conflicts of Interest

The authors declare no conflict of interest.

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