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Graphene Oxide from Improved Hummers' Method: Is This Material Suitable for Reproducible Electrochemical (Bio)Sensing?

Karel Lacina,^{1,*,z} Ondřej Kubesa,^{2,=} Veronika Horáčková,¹ Zdeněk Moravec,^{1,3} Jan Kuta,⁴ Petr Vanýsek,^{5,6,*} and Petr Skládal^{1,2}

¹CEITEC - Central European Institute of Technology, Masaryk University, Brno 625 00, Czech Republic

²Department of Biochemistry, Faculty of Science, Masaryk University, Brno 625 00, Czech Republic

³Department of Chemistry, Faculty of Science, Masaryk University, Brno 625 00, Czech Republic

⁴RECETOX - Research Centre for Toxic Compounds in the Environment, Faculty of Science, Masaryk University, Brno 625 00, Czech Republic

⁵Department of Chemistry and Biochemistry, Northern Illinois University, DeKalb, Illinois 60115-2862, USA

⁶Brno University of Technology, Department of Electrical and Electronic Technology, Brno 616 00, Czech Republic

Graphene as a superior nanomaterial is currently omnipresent and electroanalytical applications are not an exception. In this contribution, its suitability for such applications was critically assessed. We synthesized graphene oxide (GO) in five separate but identical experiments using the improved Hummers' method – well-known and extensively utilized procedure which is typically followed by various operations leading to reduced graphene oxide material. These five repeated trials were precisely controlled to keep the experimental conditions as identical as possible. The resulting five individual GO products were compared by means of elemental analysis, Raman spectroscopy, ICP-MS and AFM. The possible employment of such graphene products - electrochemically reduced GO - for electroanalytical purposes was also probed from the viewpoint of the reproducibility of modification of electrodes and the results seem disconcerting, indicating poor reproducibility. No similar study in reproducibility has been performed until now, since all previous reports always compared only different methods of preparation and only discrete experiments of preparation – repeatability has not been properly addressed.

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A flood of papers which describe almost universal utility of graphene materials including graphene oxide have been appearing since its first isolation in 2004. Diverse means of preparation and derivatization (doping) are broadly reported as well. Graphene and its related materials are being studied and employed for various applications and purposes ranging from energy storage and production, novel composites, to healthcare and electronics.¹ Extensive use for research purposes is focusing also on electrochemical sensing and biosensing. Herein, the graphene and mostly the graphene oxide (GO) are predominantly utilized for enhancement of electron transfer and enlargement of electroactive area of sensing electrodes; generally, for similar reasons as other electroactive nanomaterials are being employed. But why is graphene oxide so popular? The extraordinary popularity of graphene materials will be analyzed in the following paragraphs.

Four main methods are being employed for preparation of graphene oxide-based materials. The oldest one is typically known as the Schafhaeutl's method² (based on sulfuric and nitric acids), followed by the Brodie's method³ (introduced potassium chlorate), the Staudenmaier's "one-pot" method⁴ and finally the Hummers and Offeman method.⁵ It can be said that other current methods are basically derived from the above-mentioned ones. Probably the most significant one is the procedure reported by Marcano and Tour as the 'Improved Hummers' (IHM).⁶

Since functionalization of electrodes is still the crucial process for electrochemical (bio)sensing, it is straightforward that the graphene oxide is popular material also for this reason. Popularity of such methods probably stems from the fact that graphite is very cheap and abundant material and production of graphene with the IHM using inexpensive reagents and unsophisticated instrumentation is simple as well. The presence of various functional groups such as hydroxyl, epoxide or carboxyl residues, which provide simple modification of surfaces, also plays an important role. However, the output material is still not uniform having wide distribution of shapes, forms and dimensions, presence of defects and regions with reactive groups. Such material seems not to be suitable for generation of well-defined and well controlled processes for large-scale (industrial) modification of electrodes. The repeatability of thus generated graphene materials is

low, and the consequence is a poor control of the subsequent electrode preparation processes.

Although the Hummers' method and its improved variant are well known, brief description will not be redundant; graphene sheets originally present in the graphite material are oxidized in a three-step reaction (intercalation of acids into graphite material, transformation/oxidation into graphene oxide and penetration of water into the lattice). These oxidized graphite layers contain various functional groups such as hydroxyl, epoxide, carbonyl or carboxyl residues which are responsible for exfoliation of the separated graphene oxide sheets due to the steric and electro repulsive forces.^{7,8} These functional groups are suitable for simple modifications of surfaces without any demanding additional processes.⁹

In majority of scientific works, investigators usually develop novel materials with described breath-taking abilities. Repeatability (if considered at all) for example in electrochemistry, is usually tested only in the way that from a single batch of graphene material several electrodes are prepared and characterized. Repetitive experiments, if included at all, are performed only on the selected electrodes (typically those exhibiting the best properties. . .). However, the reproducibility of the graphene preparation itself and reproducibility of the electrode modification are usually neglected, although they are much more crucial from the viewpoint of industrial applications.

Unfortunately, repeatability of the preparation of the material is highly stochastic and random and the danger of fluctuations or introducing diverse errors is extraordinarily high. This is not the case just for morphological features of the GO but also for content of the additives or impurities which are typically introduced not only during preparation but also during purification and redispersion of GO particles.¹ Moreover, immobilization reactions and techniques for modification of electrodes, which are necessary for electroanalytical purposes, suffer from the same problems, too.

Here, we do not want to comment all possible ways of graphene preparation and all possible applications of graphene-based materials since one cannot span over all fields of expertise; we do want to comment on the electrochemical (bio)sensing with the help of graphene material prepared by oxidative exfoliation methods. However, other fields of the graphene research and application can derive implicating conclusions.

We do not intent to criticize general usage of the graphene for electrochemistry, but we want to emphasize that authors should be aware

^zThese authors contributed equally to this work.

^{*}Electrochemical Society Fellow.

^zE-mail: lacinak@chemi.muni.cz

that (reduced) GO is not the only ideal solution for all electrochemical issues and that some unexpected and overwhelming properties of such material can simply be an accident or random error from the expected average behavior (which is definitely interesting for some scientific research, but also the reproducibility and cost-effectiveness of that novel approach should be taken into account and commented). Contrary, much more detailed characterization should be performed as this material is truly very irreproducible and unpredictable. Each specific application needs some specific form of characterization – contaminating substances interfering in one particular application may not necessarily exhibit interferences or be even beneficial in other application.

Experimental

Material and chemicals.—Graphite powder, 100 mesh ($\sim 150\ \mu\text{m}$) was obtained from Alfa Aesar (www.alfa.com). Hydrochloric acid, phosphoric acid, potassium permanganate, ethanol, diethyl ether, hydrogen peroxide and potassium chloride were purchased from Penta (www.pentachemicals.eu) and sulfuric acid from Lach:ner (www.lach-ner.com). Cysteamine hydrochloride was obtained from Sigma-Aldrich (www.sigmaaldrich.com) and mica grade V-1 muscovite was purchased from SPI Supplies (www.2spi.com). Milli Q water was used throughout the experiments.

Preparation of graphene oxide.—The samples of graphene oxide were synthesized according to the Improved Hummers' method by Marcano et al.⁶ with some adjustments in acidity of the mixture, temperature and timing.¹⁰ Briefly, the mixture of 96% sulfuric acid and 85% phosphoric acid in 7:3 ratio (105/45 ml) was poured over the mixture of graphite and potassium permanganate in 1:6 ratio (1/6 g). The whole mixture was then stirred at 50°C for 15 hours (controlled by thermometer directly in the reaction mixture). The mixture was let cool down to room temperature (2 h), poured over a mixture of ice and hydrogen peroxide (200 g ice and 5 ml of 30% hydrogen peroxide) and stirred. The color of the mixture changed into bright orange. Filtration over a 300 μm sieve followed in order to remove bigger particles (similarly to IHM); after centrifugation at 8 000 rpm for 10 min, the remaining solid was washed with water (2x), 30% hydrochloric acid (2x), ethanol (2x) and finally transferred into beaker, diluted in 200 ml of diethyl ether and filtered through 0.2 μm Teflon filter under vacuum and let dry overnight under vacuum.

Thus prepared GO was then dispersed in acidified water pH 3.0, sonicated for 2 hours and centrifuged for 10 min at 6 000 rpm in order to separate multi-layered particles. The remaining gold-brownish solution was used for experiments and stored at 4°C.

Elemental analysis.—Elemental analyses (C, H, and N) were performed on the EA-1108 Analyzer (Fisons Instruments) at The Regional Centre of Advanced Technologies and Materials, Palacký University Olomouc, Czech Republic.

Inductively coupled plasma – mass spectrometry (ICP-MS).—Samples were analyzed by inductively coupled plasma mass spectrometry (Agilent 7700x ICP-MS, Agilent Technologies) after sample decomposition in microwave digestion system (MWS3+, Berghof) with concentrated HNO_3 , H_2O_2 and HCl (Merck, analytical grade chemicals). Content of P, S, K, Ca, Na, Mg, Mn and Fe was quantified by external calibration with correction on internal standards (^6Li , ^{45}Sc and ^{72}Ge). Semiquantitative analysis was also performed on samples and content of other metals (more than 50 elements were measured) was found below 20 $\mu\text{g/g}$.

Raman and FTIR spectroscopy.—Raman spectra were recorded using the micro-Raman spectrometer Horiba Labram HR Evolution with the 532 nm laser as an excitation source. Samples were analyzed in dry state on a microscope slide. FTIR measurements were performed on the single beam FT spectrometer Bruker Tensor 27 equipped by Diamond-ATR accessory device (spectral range

4000–400 cm^{-1} , resolution 4 cm^{-1} , 64 scans). All samples were measured as pure solids. Resulting spectra were processed and evaluated in OPUS 7.2 software.

Atomic force microscopy (AFM).—AFM system Dimension FastScan (Bruker) was used to evaluate topography of the prepared graphene oxide. The FastScan-A probe (Bruker) with spring constant 18 N/m and resonance frequency of cantilever 1400 kHz was used for the imaging. Samples were prepared as follows. The mica squares ($1.5 \times 1.5\ \text{cm}$) were cleaned with a two-sided adhesive tape and any remaining microparticles were removed by compressed air. A drop (5 μl) of graphene oxide solution was deposited on the freshly cleaned mica surface at room temperature. Scanning of the sample was initiated after drying of the droplet on the mica surface in air.

Electrochemical measurements.—All electrochemical measurements were carried out in 0.05 M phosphate buffer with 0.1 M KCl, pH 7.4 at room temperature. Electrochemical measurements were controlled using Autolab PGSTAT302N potentiostat/galvanostat (Metrohm) in the standard 3-electrode set-up. rGO modified gold disk (0.5 mm) embodied in glass was used as a working electrode. Platinum wire and calomel electrode (3 M KCl) were used as the counter and reference electrodes, respectively. The values of potentials are always indicated vs. this reference. All solutions were purged with nitrogen before measurement in order to prevent any oxygen interference. Cyclic voltammetry (CV) experiments were performed with the 100 $\text{mV} \cdot \text{s}^{-1}$ scan rate in the range from $-0.5\ \text{V}$ to $0.8\ \text{V}$.

Modification of the electrodes by GO was performed as reported previously.¹⁰ Briefly, the electrodes were polished to mirror-like finish using 0.3 and 0.05 μm alumina slurries and sonicated for 10 min in ethanol in order to remove any remaining particles from polishing. Electrodes were modified with cysteamine hydrochloride solution (20 mM, 2 h) and then transferred into a 0.5 $\text{mg} \cdot \text{cm}^{-3}$ solution of GO (pH 3.0) and incubated for 4 h. After modification by GO, the electrodes were electrochemically reduced using cyclic voltammetry. Three scans were carried out in the range from 0.0 to $-1.5\ \text{V}$, scan rate 50 $\text{mV} \cdot \text{s}^{-1}$, 0.5 M KCl was used as an electrolyte.

Results and Discussion

Our main goal was to investigate the repeatability and reproducibility of the preparation of the graphene oxide (GO) using the improved Hummers' method and its subsequent immobilization onto the electrodes to examine its utilization especially for electroanalytical purposes. There were some comparative reports in the published literature, but always only comparison of several types of different synthetic pathways were tested – i.e. different derivatives of the graphene are synthesized only once, and their astonishing and novel properties are studied, e. g. Refs. 11,12. No study has compared several graphene materials prepared repeatedly using an identical synthetic route.

Here, five batches of GO were prepared and characterized using six different techniques – elemental analysis, inductively coupled plasma mass spectrometry (ICP-MS), Raman spectroscopy, Fourier-transform infrared spectroscopy (FTIR), and atomic force microscopy (AFM). Its electrochemical activity toward H_2O_2 was studied by cyclic voltammetry. All the samples were prepared under strictly identical conditions to limit possible errors and inconsistencies – all samples were prepared using the same batch of reagents, time periods, and temperatures. The concentrations were maintained with the highest possible accuracy and work was done under the same laboratory conditions.

Elemental analysis gave results which are summarized in Table S1 (in Supporting Information). The contents of C, H, S elements are comparable and similar throughout the prepared samples. On the other side, the analysis with ICP-MS (Table I) revealed dramatic difference in the content of manganese and iron – quite important components from the viewpoint of an electrochemist (other impurities introduced throughout the synthetic process were comparable for all trials). Even trace amounts of these metals can dramatically affect the

Table I. ICP-MS analysis of five graphene oxide samples.

	P [mg/g]	S [mg/g]	K [mg/g]	Mn [μg/g]	Fe [μg/g]	Ca [μg/g]	Na [μg/g]	Mg [μg/g]
F0 (graphite)	< 0.002	0.07	< 0.003	0.02	< 1	< 0.4	< 0.8	< 0.1
F1	7.67	25.0	2.86	574	8	79	42.5	4.6
F2	6.84	25.9	2.67	428	1	161	26.8	4.5
F3	5.90	22.0	1.94	261	3	54	29.1	4.7
F4	5.92	21.9	2.45	204	2	60	26.0	4.0
F5	5.71	22.8	2.13	90	2	131	33.3	5.6
F3* (old)	3.70	14.7	1.07	379	7	756	46.8	29.3
F4* (old)	2.50	45.0	4.78	5090	6	253	66.4	4.3

Samples F1 – F5 were completely decomposed by concentrated acids and hydrogen peroxide in microwave digestion system however sample F0 did not properly react under the proposed conditions, so rather the liquid extract of this material was analyzed.

*Old samples from the previous study,¹⁰ where different initial graphite material was used, illustrate well that if one does not carefully pay attention to the procedure of preparation (unlike in the current study), highly different resulting materials are obtained.

electrochemical behavior of the studied material; for example, different catalytic activity of oxygen reduction was observed for rGOs with different distribution of Mn in its structure¹² and Fe exhibits catalytic activity in many diverse electrochemical processes. Differences, which one might consider as negligible (e.g. in trace amounts of iron), can have dramatic impact on the material performance. Moreover, two GO materials described in our previous study were reanalyzed using ICP-MS. Even higher difference in Mn content was observed for these samples. This is important, as the experimental procedure for the samples *F3 (old)* and *F4 (old)* was the same, however, the uniformity of the parameters of the previous synthesis¹⁰ was not as strictly controlled in comparison with the current study. The general differences in the content of the chemical elements between the new and old batch should be noted - the quality of the initial reagents (especially graphite) governs the quality of the resulting material, as well. All these data confirm the enormous influence of all the conditions during preparation.

Raman and FTIR spectroscopy.—Raman spectroscopy together with FTIR are mostly used throughout the characterization of GO materials. These exhibit characteristic maximum intensities if probed by Raman spectroscopy: **D** band around **1360 cm⁻¹**; **G** band at about **1580 cm⁻¹** and **G'**, typically referred as **2D** band at **2700 cm⁻¹**. The D band originates from the disordered carbon structure, and the G

band is related to the sp² hybridized carbon atoms. The **2D band** significantly changes according to the quality of graphene and number of its layers.^{13,14} In case of pristine graphene, the 2D band is very sharp and symmetrical, but as the number of layers increases, the 2D band becomes broader with a small shoulder oriented toward lower wavenumbers and it also slowly shifts to higher wavenumbers.¹⁵ The number of layers could be also derived from the ratio of band intensities I_{2D} to I_G and their position and shape.¹³ The relative intensities of D and G bands I_D/I_G can be also used for determination of number of disruptions in the sample.

In Figure 1A, comparison of Raman spectra for all prepared materials is provided. The baseline was subtracted from the signal and the data were normalized using height of the G peak. It is assumed that the peaks D, G and 2D are characteristic for particular structural properties^{13,16} or at least the ratio of these peaks should be the same for the materials with the same properties. However, subtle differences in the spectra could be noted in 2D region. The assumed comparability of ratios of I_G over I_{2D} is not completely valid (Table II) which means that different number of layers of graphene oxide and different structural defects are present among all samples. Moreover, the sample F1 exhibits a more apparent shoulder from 1000 to 1250 cm⁻¹ compared to the remaining samples (Figure 1A). Similar observations were evident from FTIR spectroscopy (Figure 1B). The absolute similarity is questioned after detailed inspection of the results, see different intensities of the particular bands (830, 1035 and 1224 cm⁻¹). However,

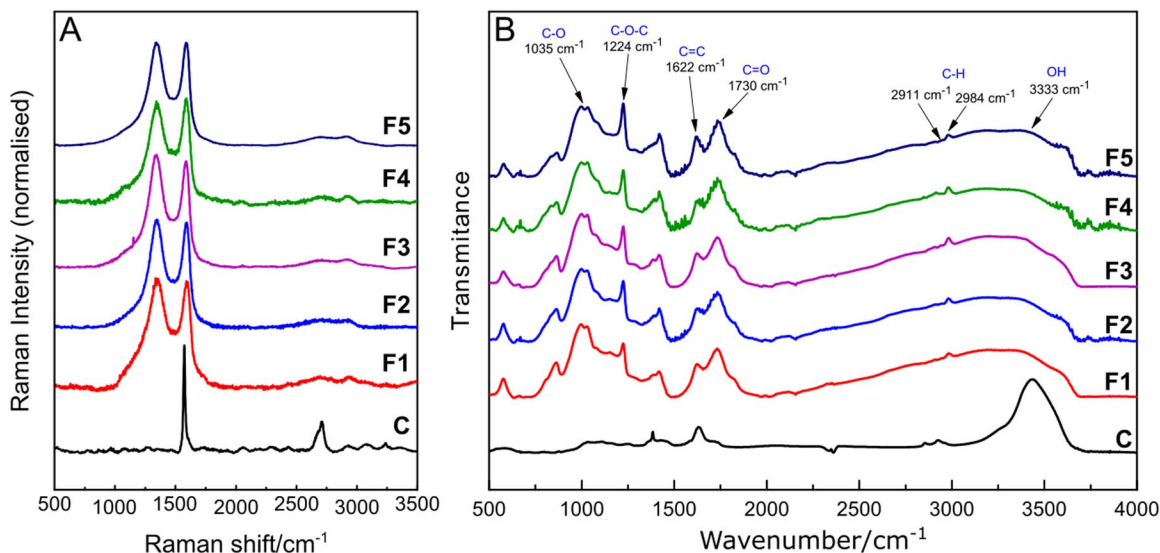


Figure 1. Raman spectra of five GO samples F1-5 and initial graphite material C. Typical bands for GO were observed. Differences in number of graphene layers are present (A). FTIR spectra of GO samples F1-5 and C – graphite used for the production of GO (B).

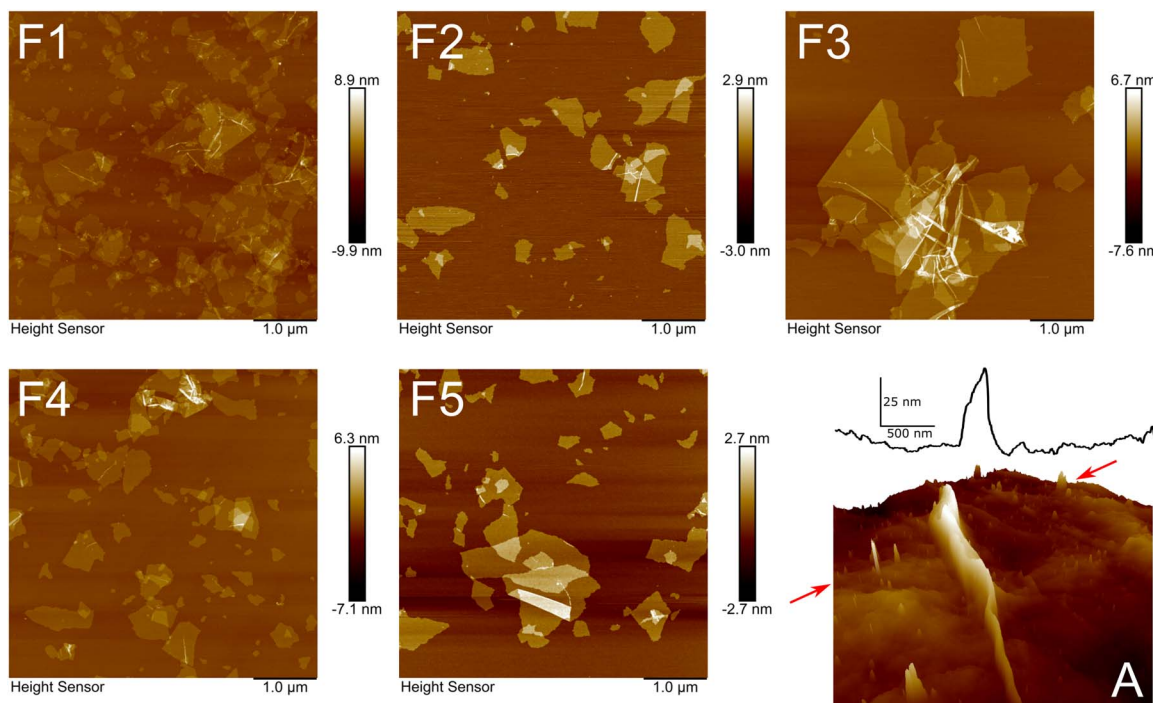


Figure 2. AFM topographies of graphene oxide on mica surface. No regularity and random distribution ranging from nm to above μm dimensions has been observed for all samples. (A) AFM scan of sample F5 performed in phosphate buffer confirming presence of sheets of rGO bound perpendicular to the electrode surface. The sheet is nearly 50 nm high. The red arrows mark the place where the cross-section was made (inset).

no significant differences amongst the prepared GO samples were visible even after careful comparison of the datasets in Figure 1; majority of differences were in the range of the usual stochastic error. So, from the viewpoint of Raman spectroscopy and FTIR, prepared GO samples were similar. This leads to the misgiving whether these methods could be used for the characterization of GO materials solely as elemental analysis found dramatic difference in the distribution of particular metals and different electroactivity upon immobilization of these GOs onto the electrode surface was observed (see further Electrochemical comparison section).

Atomic force microscopy (AFM).—AFM revealed quite random and wide range of structures of graphene oxide from tiny chips to wide micrometer-sized sheets and, what is the most important, without any regularity (Figure 2), for more AFM examples see Supp. Inf. As the GO sheets produced by IHM are oxidized predominantly on the exposed edge sites, it was anticipated¹⁰ that shorter GO sheets could be possibly “standing” on the electrode surface in a perpendicular orientation with regards to its surface. This has been confirmed on dry samples and even in an aqueous buffer solution (Figure 2A and Supp. Inf.). Herein, the electrode was activated for the graphene immobilization with cysteamine providing amine groups. Epoxy and aldehyde groups were expected to interact with the amine-modified surface of the electrode and afterwards GO was electrochemically re-

duced. Perpendicular sheets of graphene sticking out of the electrode surface to the solution were present and visualized.

Only short rGO sheets can be bound in the perpendicular manner. Wide rGO sheets will probably collapse onto the electrode surface and will be bound in parallel orientation insulating thus much bigger areas of the electrode (the “insulating” properties of the parallel sheets compared to perpendicular sheets of rGO were discussed previously¹⁰ and will be mentioned in the next section).

Electrochemical comparison.—Five electrodes were simultaneously modified with five different GO materials, one selected GO material was immobilized repeatedly onto two more electrodes and electrochemical behavior was probed. The immobilization procedure included electrochemical reduction of GOs thus giving reduced graphene oxide (rGO) on the surface of the electrode. The subsequent electrochemical experiment was performed in a manner that each electrode was inserted in the solution of H_2O_2 and cyclic voltammetry was measured. Although analogous shape of the CVs as in the previous study¹⁰ was observed and this shape was similar for all electrodes with different rGOs samples (Figure 3A), differences among measured data can be noticed after closer inspection (Figure 3B).

Difference of potential of oxidative peak between samples F5b and F3, 4 is more than 80 mV which can be hardly ascribed to the “intra-measurement” error, marked by arrows in Figure 3B. (The small spikes are ascribed to the presence of a trace amount of Ag on the surface of the electrodes. This will be addressed in the near future in a manuscript which is under preparation). Furthermore, the shape is not absolutely identical – two oxidative peaks are overlapping for F3 sample (marked as *a* and *b* in Figure 3B). The shape of reduction of H_2O_2 differs, too. The most similar are the F4 and F5a samples.

Even such small differences observed for the “same” rGO materials are usually considered in the literature as a proof of different doping, modification or any other “successful” functional manipulation with graphene oxide molecules.^{17,18} However, in our case these results originated from the same rGO samples which were

Table II. Ratios of characteristic peaks from Raman spectra.

	I_D/I_G	I_G/I_{2D}
F1	1.03	11.9
F2	1.05	13.3
F3	1.08	14.8
F4	0.97	18.8
F5	0.99	11.7
Graphite	-	3.4

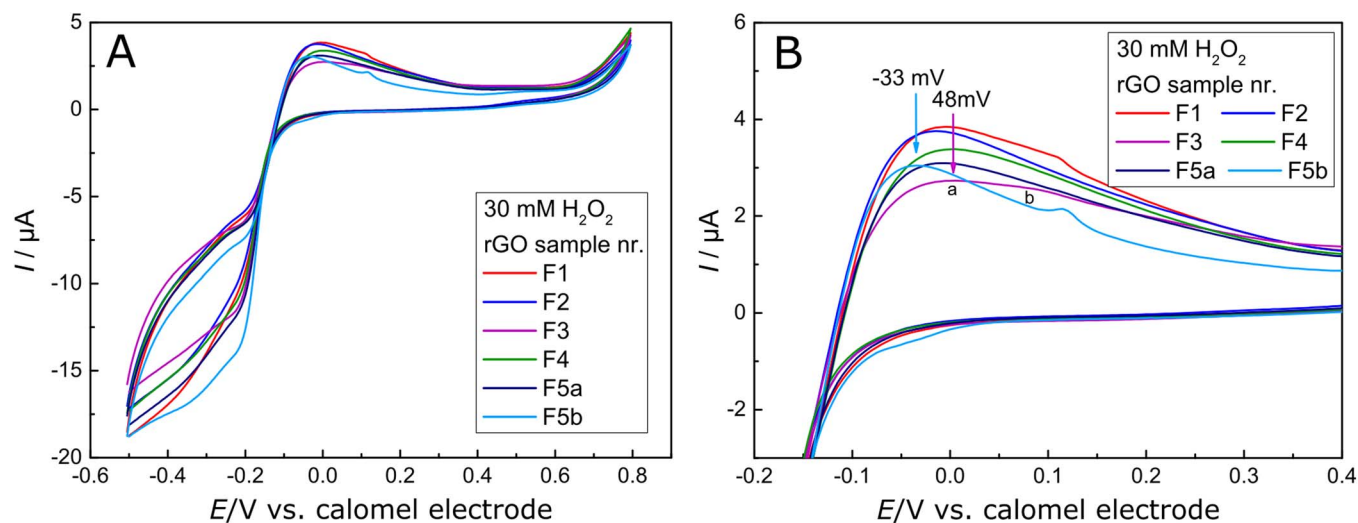


Figure 3. Cyclic voltammetry of six electrodes which were modified with F1-F5 samples of GO. Sample F5 was deposited for two-times (A). Zoomed view of the same experiment to visualize the differences in the oxidation region. Oxidative peaks of F3 (48 mV) and F5b (−33 mV) are marked by arrows. Two overlapping peaks in F3 are marked by a and b below the corresponding curve (B).

synthesized upon the identical conditions and thus should give five identical outputs.

In the following experiments, three separate electrodes were identically modified with F5 rGO sample (Figure S2). However, it was successful only in two cases, which is not attractive from the view point of its possible utilization for biosensing purposes. It should be also stated, that the procedure and number of repetitions of modification was chosen according to the most common (and most frequently published) ways for modification of electrodes. Nevertheless, oxidative peaks of H_2O_2 of electrodes modified with samples F5a and F5b differed in almost 30 mV. The differences in the experiment where rGO F5 sample was modified for three repeated times is not negligible leading to the conclusion that also the electrode modification process is random and influenced by many unknown factors controllable with difficulties.

Again, it must be noted that the stochastic processes present throughout the whole method of the GO synthesis are also present during the modification of electrodes. As it was documented by AFM, a whole range of various rGO sheets is bound on the electrode surface. Some of the sheets immobilized perpendicularly, some of them were parallel with respect to the electrode surface. As the sheets are of different dimensions and shapes, one can expect that also the different parts of electrode will be covered differently, i. e. in a random manner. If the electrode is big enough, the randomness of these processes (different coverage with different rGOs in different positions) will be averaged. However, if the electrode dimensions will be decreased to e.g. a microelectrode – the size of sheets will become comparable to dimensions of electrodes, then the manner of the rGO immobilization – in parallel or in perpendicular – will dramatically affect the resulting electrode performance. The sheets bound in parallel will “insulate” the surface and decrease the electrochemically active area of the electrode, compared to perpendicular sheets (faradaic electrochemical reactions occur on the edges and defect sites as it was previously demonstrated^{19,20} and discussed;¹⁰ term *insulation* is here understood as being less reactive compared to the basal material of the metal electrode or compared to the perpendicular sheets with edges and defects exposed to the solution). Such thought experiment can be lead to its final extreme – imagine that there is only one dimension of rGO sheets, the electrode is of the same dimensions as these sheets. The rGO sheets can be bound in two manners – first, one sheet in parallel, covering thus the whole electrode and second, bunch of sheets bound in perpendicular manner, in stack. These two electrodes would perform entirely differently – their activity could be comparable to the response of edge plane and basal plane graphite electrodes (for

illustration see Figure S3, as can be expected edge plane graphite performs much better than the basal plane electrode).

Graphene can be bound by the reactive edges and let stand erected perpendicularly to the surface of the electrode (confirmed by AFM measurement in the phosphate buffer) and at this point such electrode would perform really well. However, usage of edge plane^{19,21} graphite electrodes seems much more controllable and simpler.

If one still wants to use the IHM method for the production of GO, there should be a possibility to separate small flakes using e.g. ultracentrifugation, but in that case the costs of the synthetic process would increase and then the cost-effectiveness and simplicity of these procedures would be lost. Furthermore, the underlying material of the electrode has much higher effect on the electrochemical behavior regardless of the orientation of the rGO sheets – the underlying material governs the shape of the measured signal. This phenomenon has already been observed previously¹⁰ and is apparent also here in the experiments with H_2O_2 (compare the signals Au and glassy carbon electrodes modified with rGO, see Figs. 3 and 4 in Ref. 10).

It is clearly difficult to make any reasonable conclusions from one separate synthesis of graphene oxide in the light of presented data where even absolutely controlled repeated uniform procedure gave different results. So, we do not only emphasize the previously stressed necessity of characterization of resulting graphene materials²¹ – much higher stress should be given on repetitive preparation (synthesis of more than one batch of particular material) for possibility of averaging and generalization of the observed properties. It should also be mentioned and emphasized here that the value of the scientific result which nobody can reproduce (not even the originators/inventors of the idea) is low.

Conclusions

The main objective of this contribution was to compare five identical preparations of graphene oxide material done with the IHM. The production processes were thoroughly controlled to reach the most similar and thus comparable products. The resulting materials were characterized using elemental analysis, ICP-MS, Raman spectroscopy, FTIR, and AFM. Although FTIR and Raman spectroscopy did not find any significant irregularities, severe deviations and heterogeneities among all prepared graphene materials were described. These included, for example, different distribution of Mn and Fe elements and wide range of dimensions of graphene sheet. All these deviations severely affected the planned subsequent utilization – electrochemical analysis with rGO modified electrodes.

In that case, different electrochemical behavior of such electrodes was observed.

One can oppose that there are not so big differences in the presented data, however the experiments were designed so that all the procedures would be maximally uniform (same). And if such differences were observed upon optimal experimental conditions, one can simply ask what happens for different procedures and graphene preparations and during not so strictly controlled conditions. Another issue could be seen in that higher number of and more complex analyses should be performed (XPS, information on the chemical states of each element) and more significant statistical data should be provided to establish a robust conclusion. However, such work would be much more demanding, and our intent was not to reveal any reasons for poor reproducibility, this is far beyond the scope of this report. Our intent was predominantly to provoke scientists working in the field to think about the reliability, usefulness and applicability of the newly developed materials.

Graphene oxide was for many times reported as a material with unique properties, but usually these unique properties are not inherent for GO itself but rather the property imparted by the dopant(s). And then, GO serves only as an inert matrix for this doping (or underlying) material. Thus, much higher endeavor should be devoted to the reproducible preparation of graphene materials than thousands of more or less possible applications. Without any principal breakthrough in the area of GO production (in reproducible way), it is not possible to utilize GOs in a more consistent and practical way. Also, without any method for purification or mass production of well-defined graphene structures, the production processes based on random exfoliation of graphite will not result in reasonable applications. It is possible that even graphene prepared in this way would exhibit extraordinary performance, however, there is minimal chance of successful repetition of the final material. If there should be some reasonable application in sensor technology, the robust method for mass production of the defined materials must be introduced.

If the production and manipulation are highly controlled and performed under accurate and defined conditions, then graphene can undoubtedly be very useful material for a slew of applications²² not affected by whim of chemistry. The widely used IHM method for the generation of graphene materials is not very suitable for electroanalytical purposes or at least without any purification steps.

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ORCID

Karel Lacina  <https://orcid.org/0000-0002-3684-3902>

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