

Carbon nanoparticles: response to environmental stimuli. Graphene oxides

Final report

Crystalline carbon materials, including carbon nanotubes, graphene and their derivatives, have been in the focus of intensive studies due to their unique structural, physical and chemical properties. They are promising candidates for a wide variety of applications, e.g. within the fields of energy storage and conversion, supercapacitors or catalyst supports in fuel cells. Although graphene has proven its ability to be applied in many sectors, its hydrophobicity is often a critical factor in manufacturing. Hydrophilic graphene derivatives such as graphene oxide (GO)¹ and reduced graphene oxides (RGOs) may help to overcome these problems. For many years, GO has been mainly considered as an intermediate of wet chemical graphene production. Today, however, graphene oxide itself has gained its own well-deserved recognition and its application potential is continuously expanding. Although in the manufacture of GO-based electrodes and optoelectronic devices, GO is used in a solid, dry form, most of the processing happens in a suspended form. Direct ink writing (DIW), 3D printing, film casting, wet spinning, etc., are techniques that also require GO dispersed in liquid medium. For this reason, GO is also commercialized in the form of aqueous dispersions.

In this project the behavior of multilayer graphite oxide obtained with wet oxidative exfoliation way according to a modified Hummers method² was studied from various aspects.

The techniques employed included transmission electron microscopic imaging (TEM), thermogravimetry (TG), powder X-ray diffraction (XRD), Raman spectroscopy, attenuated total reflectance Fourier transform infrared spectroscopy (ATR-FTIR), X-ray photoelectron spectroscopy (XPS), UV-Vis spectroscopy, dynamic light scattering, zeta potential measurements, potentiometric titration and rheology. (Only preliminary neutron scattering results are reported here, as further studies were interrupted by the edipemy.) A brief summary of the results is given below.

1. Homogeneity of the GO suspension³

The exfoliated particles are heterogeneous in their physical dimensions and chemical features. Our aim was to reveal the range of these differences. Without any further treatment the particles of the as-prepared aqueous suspension were separated into five fractions by controlled centrifugation. The fractions and the parent GO were characterized and compared by the wide range of methods revealing their diversity in size, chemistry and rheological properties.

We found subtle but systematic differences exceeding the standard deviation of the applied methods in the morphology and the chemistry of the GO platelets (*Figure 1*). Despite the expectation, no systematic trend was found across the fractions in the dimensions, the number of layers, the defect concentration or the chemical composition along the fractions. This finding can be

¹ Although in literature graphene oxide and graphite oxide are not always distinguished, the material we use is **graphite oxide** according to the nomenclature recommendation of Carbon journal [A. Bianco, H.-M. Cheng, T. Enoki, Y. Gogotsi, R.H. Hurt, N. Koratkar, T. Kyotani, M. Monthieux, C.R. Park, J.M.D. Tascón, J. Zhang, All in the graphene family – A recommended nomenclature for two-dimensional carbon materials, *Carbon* 65 (2013) 1–6, <https://doi.org/10.1016/j.carbon.2013.08.038>.]

² W.S. Hummers Jr., R.E. Offeman, Preparation of graphitic oxide, *J. Am. Chem. Soc.* 80 (1958) 1339, <https://doi.org/10.1021/ja01539a017>, D.C. Marcano, D.V. Kosynkin, J.M. Berlin, A. Sinitskii, Z. Sun, A. Slesarev, et al., Improved synthesis of graphene oxide, *ACS Nano* 4 (2010) 4806–4814, <https://doi.org/10.1021/nn1006368>

³ S Farah, B. Gyarmati, J. Madarász, S. Villar-Rodil, J.M.D. Tascón, K. László: Heterogeneity of Graphite oxide particles obtained with wet oxidative exfoliation. *Journal of Molecular Liquids* 386 (2023) 122451. <https://doi.org/10.1016/j.molliq.2023.122451>

explained by the fact that the separation conditions did not meet the constraints of the Stokes's law introduced for

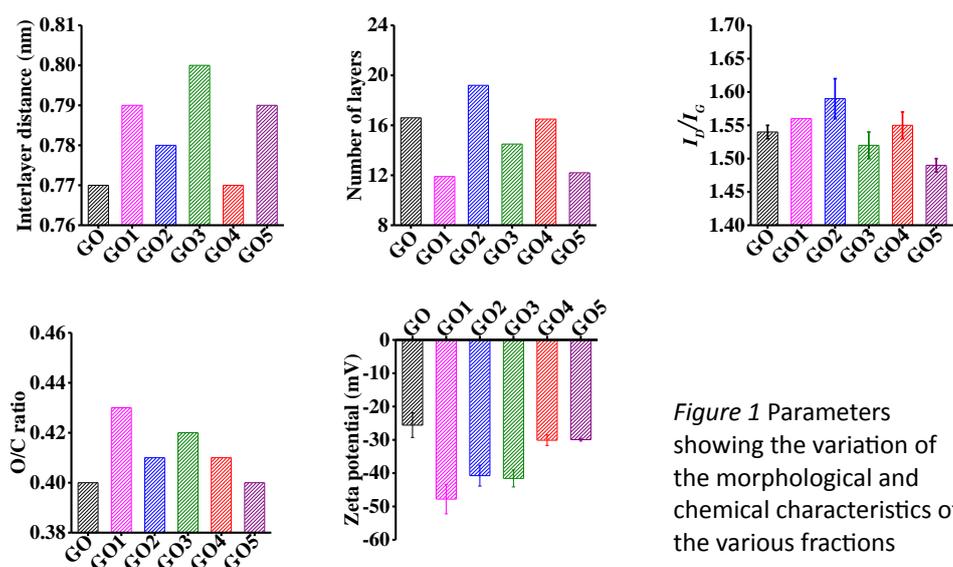


Figure 1 Parameters showing the variation of the morphological and chemical characteristics of the various fractions

the spontaneous sedimentation of fully independent solid spherical particles in a low viscosity medium where the particles get separated according to their mass. Practically none of these conditions holds here and viscosity itself shows a non-monotonic change complicating the separation. Moreover, the defects and vacancies in the particles may lead to the formation of nanocavities ready to accommodate water molecules, modifying the density of the particles. This hydration is influenced by the concentration and distribution of the functional groups along the edges of the platelets. Consequently, the separation is controlled by the density of the strongly hydrated platelets and not by the “naked” GO particles themselves resulting in non-systematic changes of the various properties across the fractions. The separation of the highly anisotropic 2D particles is therefore governed by the density of the strongly hydrated platelets, the network of which gives rise to a relatively high viscosity medium.

2. Stability of the aqueous GO suspensions on long term⁴

Although several publications comment on the alteration of graphene oxide with time, systematic works reporting these changes are few and contradictory. In order to fill this gap and get information about the shelf-life of aqueous GO suspensions, the rheological behavior of aqueous GO suspensions was investigated.

Three concentrated (10 mg/mL) aqueous GO suspensions were prepared, each with a 1-year gap. The steady shear rotational tests revealed strong shear-thinning behavior, which is explained by partial orientation of the GO flakes in the shear force field. After relaxation at low shear, the initial viscosity values were recovered, thereby implying a reversible change in the structure at moderate shear rates ($\leq 100 \text{ s}^{-1}$). The rotational tests in oscillatory mode detected a well-defined yield stress due to the GO particles touching each other and thus restricting their own mobility. The dynamic behavior of the suspensions, studied by frequency sweep tests, displayed relaxation modes of the

⁴ B. Gyarmati, S. Farah, A. Farkas, G. Sáfrán, K. László: Long-term aging of concentrated aqueous graphene oxide suspension seen by rheology and Raman spectroscopy. *Nanomaterials*, 2022, 12, 916. <https://doi.org/10.3390/nano12060916>

suspensions on different time scales. A very fast relaxation in the millisecond time range was detected, the existence of which is further substantiated by the plateau viscosity, which, in the transient shear measurements, was reached within a short time. An irreversible increase of viscosity was observed at high shear rates ($\geq 1000 \text{ s}^{-1}$) for which we observed a microstructural change and showed that is related to a process in which the highly oxidized external GO sheets are peeled off from the flakes. Both the viscosity and the dynamic moduli increase with age, particularly within the first year. The aged samples display similar shear-thinning behavior in rotational tests, and frequency-dependent dynamic moduli in oscillatory tests, but the characteristic relaxation times decrease slightly. This again suggests a microstructural change, similar to that under high-shear load. The results of the complementary Raman and XP spectroscopic studies indicate that the alteration in the rheological behavior with aging is the result of a slow oxidation process that occurs in the acidic aqueous medium during long-term storage and leads to spontaneous peeling of the external sheets from the flakes (Figure 2). Our observations draw attention to the role of storage time and mechanical load in the performance of such concentrated GO suspensions that must be considered in processing technologies and applications.

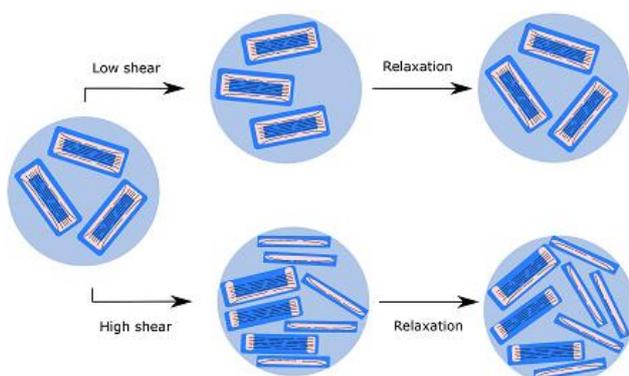


Figure 2 Proposed mechanism for microstructural changes of GO suspensions upon shearing and relaxation. Black lines represent GO platelets, pink areas strongly functionalized regions with high oxygen content, dark blue is bound water, light blue shows the bulk aqueous phase.

3. Interaction of GO in aqueous media

3.1. Interaction with small dye molecules⁵

As GO is a quasi-photostable, non-toxic, biocompatible and environment-friendly material, it has given rise to the development of many types of GO-based optical biosensors and chemical sensors. Studying the adsorption of organic dyes is a straightforward means of characterizing the binding sites on GO nanosheets. Furthermore, the results may contribute to some technical applications of GO, e.g. the cleaning of industrial waste water with GO adsorbent or the development of GO based optical sensors.

The adsorption of a fluorescent indicator dye, Oxazine 1 (OX) on GO was investigated. In the case of fluorescent dyes, fluorescence quenching that accompanies the binding to GO provides a direct method for monitoring the adsorption process (Figure 3). OX was chosen as the fluorescent probe as it absorbs in the red region of the visible spectrum, its fluorescent quantum yield is relatively high in aqueous solution, has high pKa values (same protonation form over a wide pH range). Owing to the high sensitivity of fluorescence detection, samples with low dye and low GO concentrations could be used, thus allowing to shed light primarily on the interactions of the dye

⁵ A. Paudics, S. Farah, I. Bertóti, K. László, M. Mohai, A. Szilágyi, M. Kubinyi: Fluorescence probing of binding sites on graphene oxide nanosheets with Oxazine 1 dye. Applied Surface Science 541 (2021) 148451. <https://doi.org/10.1016/j.apsusc.2020.148451>

with the strongest binding sites. The isotherms were determined at pH 6.4 and 10.2 at 20 and 35 °C and well fitted to Langmuir model. The saturation capacities corresponded to a few percent occupancy of the dissociated acidic functions of GO nanosheets by OX cations. The values for OX were lower and the adsorption equilibrium constants were one or two orders of magnitude higher than those reported for other cationic dyes on GO, measured at similar pH values and temperatures, but with higher dye concentrations. This indicates that the adsorption from the dilute solutions is governed by the strong interactions at the most active binding sites of GO. The temperature dependence of the equilibrium constants revealed that the adsorption of OX is an exothermic process. The results may contribute to the development of GO based optical sensors.

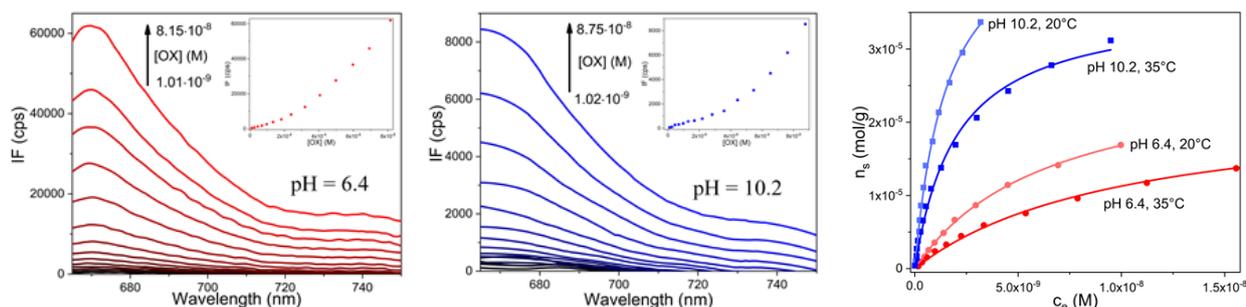


Figure 3 Concentration dependence of the fluorescence spectra of OX in aqueous GO suspensions of 2.5 mg/L at pH 6.4 and 10.2 at 20 °C. $\lambda_{\text{ex}} = 655 \text{ nm}$; and the adsorption isotherms, measured at 20 °C and 35 °C. Symbols are the measured data. The solid lines represent the hyperbolic Langmuir fit.

3.2. Interaction of GO and reduced GO with proteins⁶

The immense potential of GO and its derivatives in biomedical applications remains far from being realised. A fundamental step in biosensor preparations is to immobilise biomolecules, especially proteins, by physico-chemical means. The way in which biomolecules adsorb and migrate on solid surfaces, notably on GO derivatives, is the focus of converging interests from investigations into protein conformation, physical adsorption processes, and medical applications. Adsorption techniques yield overall information on the amount of a target molecule adsorbed in a substrate, but say little about how the molecules are distributed inside it. Non destructive techniques such small angle neutron scattering (SANS) are of particular interest for biomolecules, where the conformation is not necessarily the same in the adsorbed state as in solution. These techniques can detect the spatial structure and organisation of molecules adsorbed inside a porous medium. Our preliminary SANS and neutron spin echo measurements revealed that in GO - BSA system the protein dynamics is similar to that of the free solution (Figure 4). The BSA is hardly adsorbed on GO. On the contrary, the interaction between BSA and the hydrophobic reduced GO surface hinders the protein dynamics.

⁶ O. Czakkel, L. Sós, Cs. Weingart, E. Geissler, L. Chiappisi, Kr. László: Dynamics of proteins adsorbed on carbon nanoparticles. ILL and ESS European Users Meeting October 10-12, 2018. Grenoble, France. Poszter.

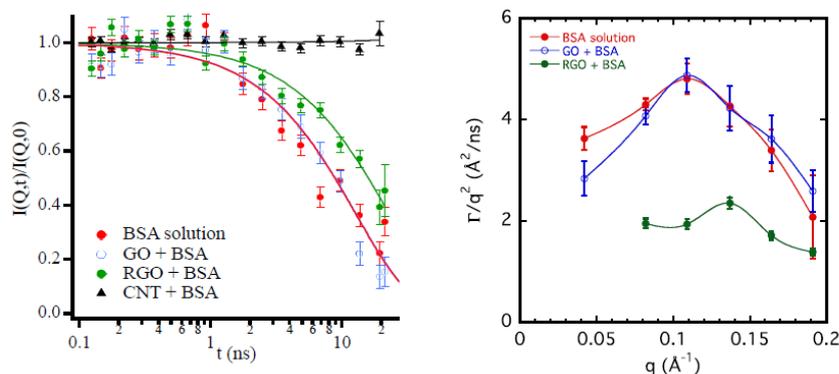


Figure 4 Neutron spin echo responses of the GO – BSA and RGO -BSA systems compared to the BSA solution

3.3. Interactions with organisms from different trophic levels⁷

The growing use of carbon nanomaterials and their mass production have raised questions about their safety and environmental impact. Despite the increasing number of studies examining their toxicity of these nanomaterials the current knowledge is still insufficient, and it is extremely important to research their potentially hazardous effects on the ecosystem in case-by-case studies.

The toxicity of GO was studied on various test organisms from diverse trophic levels (bacteria, protozoan, freshwater microbial community, plants, invertebrate animal, Figure 5) in aquatic environments, to develop predicted no effect environmental concentration (PNEC) values, and to characterize the potential environmental risk.

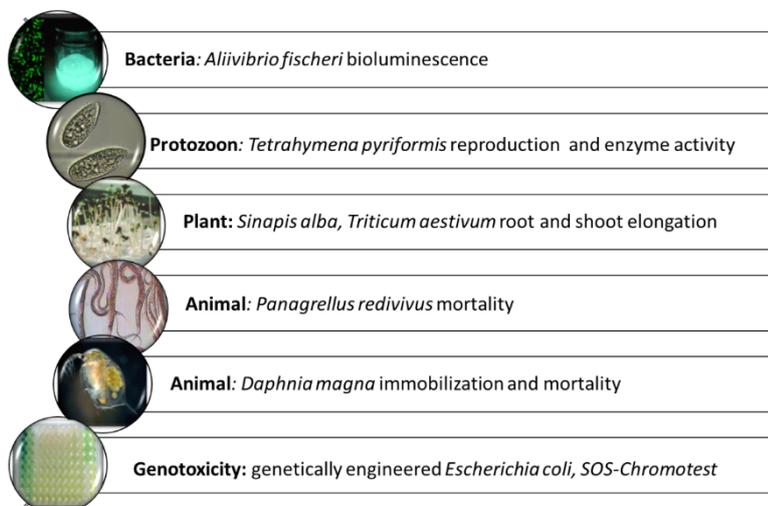


Figure 5 The developed ecotoxicological methodology applying single species tests for the assessment of graphene oxide

Beside conventional ecotoxicological endpoints (lethality, immobilization) more sensitive, sublethal endpoints (heartbeat rate, feeding activity, and reactive oxygen species (ROS)) production were determined. The possible normalization of the heartbeat rate and feeding activity in clean test medium was also investigated. The results clearly demonstrated that the reliable characterization of the effect of GO on the aquatic ecosystem requires a complex ecotoxicity test-battery including organisms from different trophic levels and with various exposure routes. The toxicity level of the

⁷ I. Fekete-Kertész, K. László, Cs. Terebesi, B. Gyarmati, S. Farah, R. Marton Rita, M. Molnár: Ecotoxicity Assessment of Graphene Oxide by *Daphnia magna* through a Multimarker Approach from the Molecular to the Physiological Level including Behavioral Changes. *Nanomaterials* 10(10), 2048 (2020). <https://doi.org/10.3390/nano10102048>; Fekete-Kertész, I.; László, K.; Molnár, M. Towards Understanding the Factors behind the Limited Integration of Multispecies Ecotoxicity Assessment in Environmental Risk Characterisation of Graphene Family Graphene-family materials — A Bibliometric Review. *C: JOURNAL OF CARBON RESEARCH* 9(4) 90, 2023. <https://doi.org/10.3390/c9040090>; I. Németh, K. László, A. Bulátkó, Em. Vaszita, M. Molnár: Ecotoxicity assessment of graphene oxides with test organisms from hierarchical trophic levels to evaluate their potential environmental risk. *Nanomaterials*, under review

tested GO depended on the exposure route, dose, duration and varied for the different test organism. The applied ecotoxicity toolkit was simple, inexpensive, multitrophic and included a wide-spectrum response to GO; besides, it delivered the results quickly (≤ 3 days) and satisfactorily. Our study demonstrated that careful selection of the bioassays in the design of a test-battery is of utmost importance in environmental risk assessment. The *Tetrahymena pyriformis* ecotoxicity test characterizing GO-mediated effect on reproduction and enzyme activity can be reliably used for impact assessment of GOs, even as an early warning system, since its effective concentration is close to the higher GO concentrations which can be measured in the environment.

4. Modification of GO

4.1. Reduction

Graphene-like properties can be partially recovered by the reduction of GO. Reduction can be performed either by thermal treatment in inert or reductive atmosphere or by wet chemical ways. The chemistry and the properties of the RGO depend on the reduction path, i.e., the temperature, duration, reducing agent, concentration, etc.⁸(Figure 6).

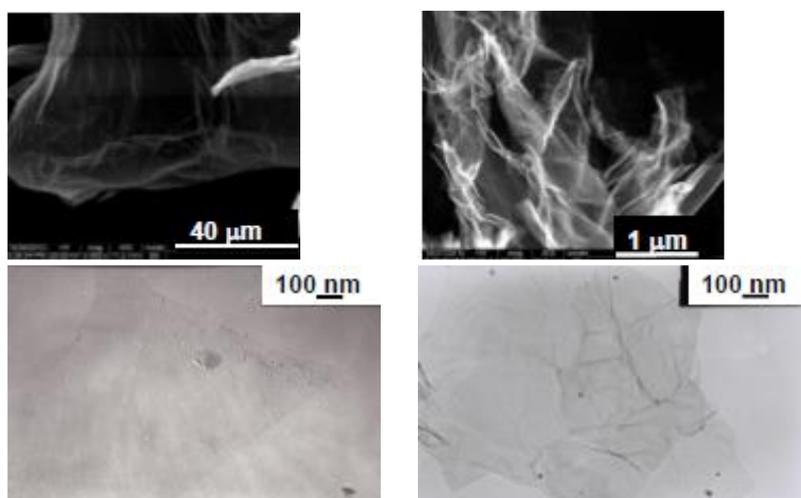


Figure 6 TEM image of GO (left) and RGO (right) at various scales

Our computational investigations were focused on the reactions of the epoxide groups on a GO surface with ammonia, water and possible reducing agents such as environmentally friendly ascorbic acid (AA) and hydrazine. (Although hydrazine is toxic and explosive, it has high efficiency and produces graphene materials with good qualities.) Firstly, periodic density functional theory (DFT) calculations were performed to identify the most stable multi-epoxide group formations for different O/C ratios. These results were used to create and verify a four-epoxide group containing finite-sized GO model to investigate the adsorption of ammonia and water on the GO flakes. The results⁹ revealed that the surrounding medium plays a crucial role in the ammonia addition, as the reaction goes through a different pathway involving a trimolecular state during the nucleophilic addition when an explicit water molecule is present. The possible deoxygenation mechanisms of an epoxide group with AA using epoxy-benzene molecule as a surface model was studied under various

⁸ S. Farah, A. Farkas, J. Madarász, K. László: Comparison of thermally and chemically reduced graphene oxides by thermal analysis and Raman spectroscopy. *Journal of Thermal Analysis and Calorimetry* 142(1), 331-337 (2020)

⁹ E. Makkos, D. Bodrogi, D. Szieberth, Computational modelling of ammonia addition on partially reduced graphene oxide flakes. *Phys. Chem. Chem. Phys.*, 2021, 23, 24738, DOI: 10.1039/d1cp02320a

conditions (temperature, aqueous media, dispersion). A feasible reaction pathway for AA is yet to be found. GO reduction with hydrazine was modelled not only with epoxy-benzene but on the four-epoxy finite size GO model to explore the possible reaction mechanisms in a multi-epoxide environment. Three different deoxygenation pathways were identified, which might simultaneously act on the GO surface, resulting in a final product in which repaired sp² domains, regions with hydrogen-bonded hydroxyl groups, and nitrogen-containing basal or edge groups coexist¹⁰.

For experimental studies thermally and chemically reduced RGOs (TRGO and CRGO, respectively) were obtained from GO by thermal (300 °C in argon flow) and wet chemical routes (using AA in basic medium). Comparison of TRGO and CRGO revealed that although the C/O ratio improves during both treatments, the reduced derivatives are different according to their chemical and thermal properties. The chemical reductive treatment caused further imperfections by the simultaneous fragmentation of the platelets.

The effect of GO and RGO was also studied in their composites with soft gels¹¹, metal organic frameworks¹² and biomass based carbon xerogels¹³.

4.2. Nitrogen implantation into GO and RGOs using radio frequency (RF) plasma

Experimental and theoretical studies showed that nitrogen doping can modulate the band structure of graphene and lead to a metal – semiconductor transition and it was also demonstrated that nitrogen doping effectively improved both the microstructure and electrochemical properties of carbonaceous materials.

The solvent-free RF nitrogen plasma treatment was applied to incorporate nitrogen atoms in relatively high concentration¹⁴. GO and the two reduced GOs (TRGO and CRGO) of different O-content were used as target substrates to reveal the influence of the functional groups decorating the graphene lattice on the quantity and quality of nitrogen incorporation. Despite the relatively high oxygen content of the samples no N–O bonds develop but three kinds of different N–C bonds of very similar concentration were formed. Reduction of the GO removed the O-groups but did not heal the vacancies where N doping may occur, however, the presence of C–O groups promotes the N incorporation. Reduction of the GO may remove the O-groups but does not heal the vacancies where N doping may happen effectively. After two days the implanted N content drops, the N1 state (sp² N in pyridine ring, C–N–C) showing the least stability.

¹⁰ Nguyen Tri Hieu, Dénes Szieberth, Eszter Makkos: Exploring the Hydrazine Reduction Mechanism in a Multi-Epoxy Environment with DFT Calculations. under review in Phys. Chem. Chem. Phys.

¹¹ O. Czakkel, B. Berke, K. László: Effect of graphene-derivatives on the responsivity of PNIPAM-based thermosensitive nanocomposites - A review. European Polymer Journal 116, 106-116 (2019). <https://doi.org/10.1016/j.eurpolymj.2019.04.004>

¹² A. Domán, Sz. Klébert, J. Madarász, Gy. Sáfrán, Y. Wang, K. László: Graphene Oxide Protected Copper Benzene-1,3,5-Tricarboxylate for Clean Energy Gas Adsorption. Nanomaterials 10(6) 1182 (2020) <https://doi.org/10.3390/nano10061182>; A. Domán, J. Madarász, Gy. Sáfrán, Y. Wang, K. László: Copper benzene-1,3,5-tricarboxylate (HKUST-1) – graphene oxide pellets for methane adsorption. Microporous and Mesoporous Materials, 316, 110498 (2021) [10.1016/j.micromeso.2021.110948](https://doi.org/10.1016/j.micromeso.2021.110948)

¹³ S. K. S. Andrade, S. S. Lakshmi, I. Bakos, Sz. Klébert, R. Kun, M. Mohai, B. Nagy, K. László: The Influence of Reduced Graphene Oxide on the Texture and Chemistry of N,S-Doped Porous Carbon. Implications for Electrocatalytic and Energy Storage Applications, Nanomaterials 2023, 13(16), 2364, 2023. <https://doi.org/10.3390/nano13162364>; S. K. S. Andrade, A. Menyhárd, Sz. Klébert, M. Mohai, B. Nagy, K. László: Effect of carbon nanoparticles on the porous texture of t-carrageenan based N doped nanostructured porous carbons and implications for gas phase applications., C JOURNAL OF CARBON RESEARCH 2023, 9, 68., 2023. <https://doi.org/10.3390/c9030068>

¹⁴ I. Bertóti, S. Farah, A. Bulátkó, A. Farkas, J. Madarász, M. Mohai, Gy. Sáfrán, K. László: Nitrogen implantation into graphene oxide and reduced graphene oxides using radio frequency plasma treatment in microscale. Carbon 199 (2022) 415-423. <https://doi.org/10.1016/j.carbon.2022.08.024>

Figure 7 compares the stability of the initial and RF treated GOs (GO N, TRGO n and CRGO N) before and after being used as an electrode in an alkalic oxygen reduction reaction (ORR)¹⁵.

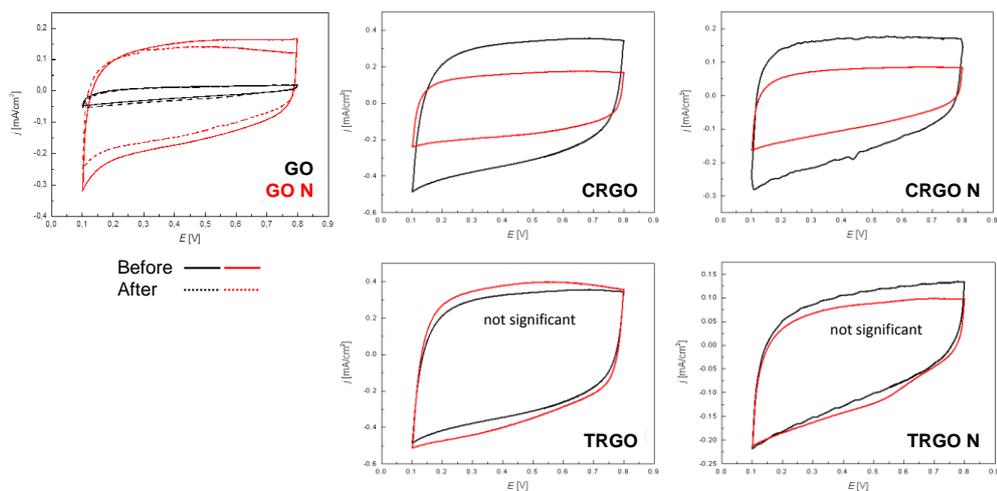


Figure 7 Cyclic voltammery curves of the pristine and plasma treated samples before and after oxygen reduction reaction (ORR) (for RGO samples black: before, red: after ORR)

¹⁵ M. Mohai, A. Bulátkó, Á. Ganyecz, M. Kállay, J. Madarász, I. Bakos, K. László: Nitrogen Implantation to Graphene Oxides: a RF Plasma Treatment and Computational Approach – Implications for Electrocatalytic Application. CESEP2023 24-28 September, Budapest, Hungary. Poszter