SZAKMAI ZÁRÓJELENTÉS / FINAL REPORT

NKFI-FK/124851

Project information

Title: Carboxylated nanomaterial dispersions for biomedical application
PI: Dr Tamas Szabo
Duration: 2017-09-01 to 2022-11-30 (63 months)

Introduction

Within the present proposal we aimed to prepare liquid-phase mixtures (dispersions) of nanometric solid particles that carry specific chemical functional (carboxylic) groups and to investigate their application, primarily in biomedicine. Two different kinds of inorganic materials – superparamagnetic iron oxide (SPIO) nanoparticles (NPs) and the oxidized form of the elementary graphite layer, graphene oxide (GO), along with chemically reduced GO (RGO) – has been considered in pristine form and in their nanocomposites.

In accordance with the original research plan, the work was divided into two separate directions, but later the results allowed us to extend the scope and apart of the realm of biomedicine, we demonstrated the use of carboxylated material nanocomposites in other fields as well. The first direction was the formulation of graphene oxide and surface modified SPIO nanoparticles and their aqueous composite dispersions for advanced magnetic hyperthermia application towards selective heat destruction of cancer cells. The properties of recently developed formulations bear shortage of quality in terms of colloidal stability and heating performance. However, we managed to achieve decent advancements both in colloidal stabilization and in specific heating rates by alternating current (AC) generated magnetic fields.

Regarding the second direction, we aimed to synthesize bioactive graphite oxide intercalation complexes for stimulated and sustained biomolecule (drug and protein) release. We relied on the exfoliation characteristics of GO which enables indirect intercalation of bioactive molecules, opening up a novel immobilization pathway for formulating GO-derived advanced drug delivery materials. Our experiments were successful only for cationic small molecules, except for proteins that have high affinity to adsorb also on like-charged carbon surfaces. The formed intercalation structures were characterized, and their interlayer expansion was precisely determined.

Finally, our goal was to demonstrate the utility of GO(RGO) as fillers or matrix counterparts in nanocomposites other than SPIO/(R)GO. We found that heterocoagulation or hydrothermal methods are complementary to each other in obtaining such composite materials for virtually all kinds of applications that either involves carbon or (metal)oxide materials. Specifically, the use and role of GO for the improvement of material properties in (i) catalysis, (ii) environmental technology and (iii) electrochemical energy storage have been demonstrated.

Progress of the research

In the beginning, we synthesized the basic materials for the project (GO and SPIO), purified and characterized them using already established protocols. These samples were used then for various types of nanocomposite formulation. Later, two students also investigated possibilities for changing the synthesis methods (for Brodie-GO, *M. Hancsárik, project work*) and obtaining new materials (nickel ferrites, *A. Vas BSc thesis*). Pure water and saline solutions were used to prepare processable GO dispersions. Time-resolved dynamic light scattering experiments were performed extensively and their pH-dependent aggregation behaviour was studied extensively, resulting in a paper related to their colloidal stability (*Szabo et al. Carbon, 2020*). Regarding IONPs, they were also extensively characterized (TEM, XRD, FT-IR) and especially their magnetic characterization by SQUID quantum interference magnetometry in collaboration with Palacký University (Czechia).

In the next stage, the ageing of the "naked" SPIO NPs obtained by standard co-precipitation has been intensified using ultrasound-assisted hydrothermal treatment at 80 °C, to foster the hyperthermally more efficient multicore cluster formation. Magnetic nanoparticles were also synthesized from different iron precursors in the presence of graphene oxide lamellae by in situ crystallization. The nanocomposites were also subjected to ageing but not at the optimal temperature of hydrothermal treatment because we observed thermal degradation of GO. Ultrasound was also omitted to avoid the disintegration of the nanocomposite structure. However, we successfully elaborated a novel cluster formation pathway using the accumulation of the primary iron oxide NPs at the GO/aqueous dispersion medium interface by heteroaggregation. This method requires only ambient lab temperature and "undamaging" conditions for the preservation of the GO structure, with other benefits such as an easily variable and wide composition range of the GO/SPIO NP composite phases (*Szabo et al. Adv Mater Sci Eng, 2018*).

Hereinafter, we investigated how the does the heat production behavior of the GO/SPIO composites change upon the modification of the oxidation degree of the carbonaceous host. Chemical reduction by $NaBH_4$ and L-ascorbic acid (LAA) under acidic (pH ~ 3.5) and alkaline conditions (pH ~ 9.3) produce different materials: alkaline reduction at higher GO loadings leads to doubled heat production (*Illés et al. Nanomaterials, 2020*). Although the treatment with $NaBH_4$ also increased the hyperthermic efficiency of aqueous GO/SPIO nanocomposite suspensions, it caused a drastic decline in their colloidal stability. Therefore, we investigated the effect of polycarboxylate coatings (PGA, PAM, PEG-copolymers) on the aggregation behaviour and hyperthermic efficiency of the nanocomposites. We have found that the appropriate coating not only improves the stability and thus the shelf-life of the product, but it also improves the heat production. This problem was a challenging goal to reach in the past three years, but we finally reached this essential milestone of the project. We found also that the colloidal stabilization mutually influences the magnetic properties of the bare NPs (*Illés et al. Nanoscale, submitted, 2023*).

Human serum albumin (HSA) is the most abundant protein in human blood plasma and its interaction with graphene materials is vital to be understood if their particles are introduced into blood stream e.g., for drug delivery applications or as composite counterparts for magnetic thermo-therapy. We found that serum proteins (HSA and BSA) feature basically the same type of adsorption isotherm (reflecting high-affinity sorption) and quantitatively similar extent of adsorption capacity, which exceeds well the theoretical monolayer capacity assuming hard-sphere protein conformations. This important finding indicates a non-conventional binding process relying on non-electrostatic components and the main type of interaction between the particles and protein molecules resembles on the hydrophobic effect resulting in the formation of hydrogel structures similar to those of polymer-induced flocculation of lamellar materials such as smectite clays.

Our national and international cooperations prompted our efforts to formulate nanocomposites of GO-derived carbon and test their utility in various applications. Originally, these composites were only considered in a photocatalytic test reaction, as part of a side project with the AKI Institute of the Hungarian Academy of Sciences (HAS). However, almost in each year we could gather enough results to publish a paper. The GO and RGO materials demonstrated different roles in the respective catalyst under operation: (i) promoter in the photocatalytic methanol reformation reaction of methanol (*Majrik et al. Top Catal, 2018)* and (ii) support material for Pd and Rh complexes used in semihydrogenation (*Liprandi et al. Mater Sci Eng Int J, 2020*) and Heck coupling reactions (*Mastalír et al. Appl Organometallic Chem, 2020*). We also synthesized and characterized GO derived TiO_2-carbon composites as electrocatalyst support materials (*Ayyubov et al. Top Catal, 2021).*

Other directions towards functional carbon nanocomposite formulation resulted in zirconium/amino acid complex and also sulfur containing materials. The former represents an entirely new class of materials that were synthesized and studied for the first time for the decontamination of fluoride ion containing aqueous solutions (*González-Aguiñaga et al. Int J Environ Res Public Health, 2022*). The latter, S-C composites were found to be efficient and ultra-stable

cathodes for Li-S batteries (*Shankar et al. Mater Today Chem, 2022*). Finally, in the last 18 months of the project we devoted a lot of efforts to write a review article, together with a Malaysian group, on the current state-of-the-art and energetic perspectives of waste-derived graphene composites (*Ikram et al. Nanotechnology Rev, under revision*).

Problems emerged during the research and their mitigation

The core of the project and the basis of the experimental work were two materials: graphene oxide and superparamagnetic iron oxides. Both needed to be synthesized already at the beginning of the project. Commercially available specimens are available for both GO and SPIO. However, we chose not to purchase them and use our own products, even if it was more time consuming to have them ready. In this way, we efficiently managed reproducibility, purity, and standardization problems. Part of these problems emerged at the early stages of the project: 1) purity of compound from different producers and (2) product uniformity of GO was not sufficient when oxidized from large-flake graphite samples). Also, we think that many potential (such as standardization) problems could be avoided in advance, and this saved time, efforts and project money.

Owing to the CoViD lockdown of the university for students, and unsuccessful attempts to recruit a new researcher to join the project, we were unable to perform many of the experiments on due time, and this eventually resulted in the postponement of the project closure date. The extension of the project helped us to finish those experiments which were reasonably achievable and we had published or have publishable results in all subfields specified in the work plan.

An interesting case was related to serum albumin intercalation into GO when simultaneous thin film deposition was necessary for producing ultrafiltration membranes. It took unusually long time and efforts (trying half a dozen of different methodoligies) to manage uniform layer deposition with one of our MSc students (*P. Gyenes, MSc thesis*).

Changes in the personnel and their influence on the progress of research

Throughout the whole project, there was a significant rotation both in the staff responsible for the experimental work and among the students. We planned to employ a researcher in full time, for the duration of four years. Instead, initially we managed to employ one researcher (Erzsébet Illés Mrs. Nyerges) for 26 months, and another for 1 month (Rita Szabó). Therefore, we needed to rely heavily on the contribution of undergraduate students (5 MSc and 5 BSc students). Unfortunately, all of the MSc students left the group after graduation. Regarding BSc thesis students, two of them are still active in the group and continue as MSc students. Fortunately, we could hire two young researchers (pharmacist Hala Roumia from Syria, and Kadosa Sajdik from Hungary) for 10 and 12 months, in order to complete the experimental part of the research. Mr Sajdik also continues to work in the lab as a PhD student. The fact that the majority of the participants needed to be trained and worked for relatively short time greatly hindered the progress of research, especially in its middle stages incl. the restriction of the "CoViD times", eventually leading to a request to extend the duration of the project. Despite this, we consider the project as successful, based on the achievements as detailed in the next point.

Achievements of the project

A) Material achievements

The financial support allowed the purchase of chemicals and equipment (UV-visible spectrophotometer, precision balance, incubator-shaker, digital magnetic stirrer multiposition plates) which were not only important for the successful realization of many experiments but enabled the establishment of the core of a laboratory ("Colloid Layers Lab") at the Institute of Chemistry, University of Szeged. The rest of investments enabled to upgrade a part of the laboratory and computer infrastructure (monitors, peripheries, notebook).

B) Publications

According to the work plan, we expected the publication of 10-12 research papers and 10-12 conference contributions. We failed to publish all results of the project within the time frame of the project and until the submission of the final report. With the publication of the remaining results, the expectation regarding the number of scientific publications will be fulfilled (10 publications incl. 1 book chapter already published as of 2022 December, with sum of IF = 36.78 + 2 papers under review in Q1 and D1 journals), and even be exceeded with 2-4 more papers, the manuscript of which has not yet been written. We have already exceeded the number of foreseen conference contributions (36 presentations as of 2022 December). Also, during the timespan of this project (in which the PI has 0.5 FTE/year), the PI published other 6 papers that are not relevant to this project (sum of IF = 21.97). Two papers published in 2020 gained already a relatively high number (50+ each) of citations. Also, two review papers have been written (one regarding IONPs already published and one about graphene materials is under review).

	Dissemination (total)	*Dissemination (item/year)*	*Quality (SJR / Impact Factor)*
Papers published	10	1.90	Q1 / 5.076 Q1 / 4.614 Q2 / 2.193 D1 / 9.594 Q4 /0 Q2 / 4.105 D1 / 7.613 Q1 / 2.321 Q2 / 1.265 N/A (book chapter)
Papers under review	2		Q1 / 6.739 D1 / 8.307
Manuscripts under prep.[1]	2		Q1 expected
PAPERS SUBTOTAL	14	2.67	N/A
Poster presentations			
intl. conf. abroad	6	1.14	N/A
intl. conf. Hungary	8	1.53	best poster prize
POSTERS SUBTOTAL	14	2.67	
	Dissemination (total)	*Dissemination (item/FTE/year)*	*Quality (SJR / Impact Factor)*
Oral presentations			
intl. conf. abroad	16	3.05	regular
intl. conf. Hungary	6	1.14	regular
ORAL PRES. SUBTOTAL	22	4.19	
Dissertations			
B.Sc. theses	5		N/A
M.Sc. theses	5		N/A
THESES SUBTOTAL	10		

[1] We consider „manuscripts under preparation", which already have full dataset (>95% of data are already collected), the manuscript was conceptualized, the experimental part is written and at least parts of display items are prepared.

C) Other achievements

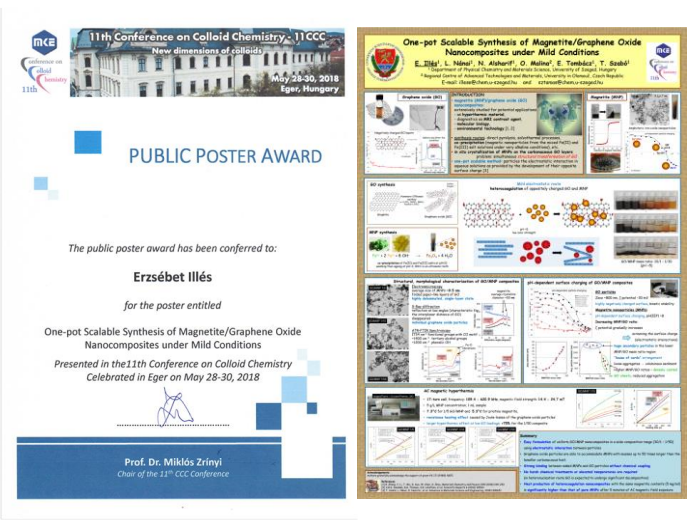
The project resulted in the extension of the international collaboration potential of the PI's laboratory. The PI undertook a short-term scientific mission in the advanced structural characterization laboratory of Prof. M. Enachescu, at the Politechnica Bucharest, Romania. Likewise, existing ties with partner laboratories at Umeå University, Sweden and Politecnico di Torino, Italy, were strengthened. Joint papers were published with a research group in Argentina and another one with Mexican partners, and one review paper jointly authored with a Malaysian group is under the second phase of the review process. A Czech scientist contributed to the magnetic characterization of our samples and coauthors a publication.

The project funding allowed us to participate in a decent number of conferences. An extra benefit of conference presence (apart from regular dissemination and learning) was that the PI could give an in-person bidding in ECIS-2019 for the next European Student Colloid Conference in Leuven, Belgium. The bidding was successful, which allowed the lab (with the members of the Momentum Biocolloids Research Group) to organize the event in 2022, after CoViD postponement and cancellation threats from the current nearby war situation. The lab's students disseminated project results extensively (3 contributions). The event contributed to the survival of the European Student Conference series in our research field and allowed for the training of European PhD students (including the local Junior Team) by an international (Hungarian, European and a US membered) Teacher Team. Details at: https://escconf2022.mke.org.hu/.

Tamas Szabo was one of the oral presenters and one of the chairmen at the e-Conference entitled as "Colloids Geneva", April 8-9th, 2021. The conference was organized for the retirement of his former postdoc supervisor, Prof. Michal Borkovec @Uni Geneva, who has been one of the leading scientists in colloid and interface science. Therefore, the results of this FK project gained great visibility among the global leading scientists in this area.

The PI was the guest editor of the open access journal "Nanomaterials" (MDPI publisher (ISSN 2079-4991), Q1 SciMago journal ranking, IF = 5.076) Special Issue entitled as "Synthesis and Applications of Graphite Oxide and Graphene Oxide Nanocomposites". The Special Issue has been completed with 14 published articles (incl. 1 review). We also published therein a project-relevant paper on magnetic hyperthermia properties of RGO/MNP composites. More details at: https://www.mdpi.com/journal/nanomaterials/special_issues/graphite_graphene_nano/.

Another remarkable achievement is the "Best poster" prize authored by E. Illés et al. from "11th Conference on Colloid Chemistry". This congress was an international meeting of the researchers who are expert in the field of colloid and interface science. This poster award was granted on the basis of public votes of the conference participants, and we think that colleagues broadly accepted this piece of research positively.



Finally, the training aspects of the project were noteworthy. Upon the five years, seven undergraduate students have participated in the research programme during summer periods and gained important professional skills. A total of 10 theses are in relation of this project; 5 MSc theses (Árpád Turcsányi, Nizar Alsharif, Balázs Barna, Lajos Domokos, Péter Gyenes) and 5 BSc theses (Mária Gregus, Anna Vas, Boglárka Gábor, Anett Németh, Virág Konkoly) were successfully submitted and defended. One student will submit the MSc thesis in May 2023. International students also visited the lab: José AP Tavares (Univ Guadalajara, Mexico) for one month and Ljubov Kulyabko from the Moscow Aviation Institute spent 2 months with us.

Progress of the project, milestones

- **2017 September: project kick-off**
- 2017 Oct: Erzsébet Illés (EI) joins the lab which is established under the informal name "Colloid Layers Lab"
- 2018 Jan: the two largest-scale investment of the project, a spectrophotometer equipped with a film sample holder and an incubated orbital shaker has been purchased
- 2018 March: Tamas Szabo (TS), EI and Etelka Tombácz (ET) participates in the COST – RADIOMAG Annual Action Progress Conference &MC meeting; TS's oral presentation
- 2018 April: EI's COST Short Term Scientific Mission @ Demokritos Research Centre in Athens
- 2018 May: ET, IE, TS and students participate at the 11th Conference on Colloid Chemistry" conference with 3 posters and one talk. TS contributes also as the secretary of the conference, while ET was one of the chairmen of the event.
- 2018 July: IE is an invited lecturer in „Women in Science" section at „FEMS Junior EUROMAT 2018 The Main Event for Young Materials Scientists" conference in Budapest

- **2018 September: start-up of the second year of the project**
- 2018 September: participation of two project members (EI, TS) at the European Colloid and Interface Science (ECIS2019) conference in Ljubljana
- 2018 October: José AP Tavares (Univ Guadalajara, Mexico) visits the lab for one month using the grant of Tempus Public Foundation
- 2018 October: T. Szabó participates at 8th SIWAN Szeged and presents poster with former MSc student N. Alsharif and with JAP Tavares giving oral presentation
- 2018 November: a chapter in the book entitled „Titration: Theory, Types, Techniques and Uses" (Ed. T.L. Joshi, Nova Publishers) is published on colloid titrations by E. Tombácz et al.
- 2019 March: T Szabó is at the „Aveiro spring meeting" of the COST Action „Multicomp CA15107"
- 2019 April: three group members participate at the final meeting of the MAGBIOVIN project entitled „Magnetic nanoparticles and their applications in medicine" (Vinca Institute, Belgrade)
- 2019 June: four group members participated at the CPBCI conference (2-6 June, Eger) and held 3 oral and 1 poster presentations
- 2019 June-July: Ljubov Kulyabko from the Moscow Aviation Institute arrived to the lab and stays for 2 months using the Training Grant of IHSS
- 2019 June-July: T. Szabó undertakes a Short Term Scientific Mission at the Politecnica Bucuresti (Bucharest, Romania) in the frame of COST Action „Multicomp CA15107"

- **2019 September: start-up of the third year of the project**
- 2019 September: EI and TS participate at the European Colloid and Interface Science (ECIS2019) conference in Leuven, Belgium, with scientific oral and bidding presentations
- 2019 December: the only collaborative, review-type paper was accepted in the OA journal "Magnetochemistry"
- 2020 March-September: dissemination of results slowed down because three conference participations cancelled due to CoViD19, and student lab work also completely ceased for ca. half year due to restrictions
- 2020 July: Tamas Szabo is one of the oral presenters at the International e-Conference on Advanced Materials and Future Challenges

- **2020 September: start-up of the fourth year of the project**
- 2020 September: TS holds an oral presentation at the hybrid final meeting of the COST Action "Multicomp" (September 24-25) Slovenj Gradec, Slovenia
- 2020 October: TS presents a talk on the virtual online conference and expo, GRAPHENE 2020, on size-dependent aggregation of GO
- 2020 November: Hala Roumia joins the project as a research assistant
- 2021 February: scientific contact was initiated with Dr. Robert Kun at the HAS AKI Institute on the development of functional GO-derived composites for battery applications with first experiments performed in that month
- 2021 February: Tamas Szabo holds an oral presentation at the fully virtual conference CCE-2021, San Francisco, USA, on the perspectives of graphene oxide derivatives in catalytic processes
- 2021 March: a scientific collaboration was initiated with Dr Rabia Ikram @ University of Malaya on waste-derived carbon materials
- 2021 April: Tamas Szabo is one of the oral presenters and one of the chairmen at the e-Conference entitled as "Colloids Geneva".

- **2021 September: start-up of the last year of the project**
- 2021 September: TS and PhD student Peter Nagy (PN) participate at the Interfaces 2021 Conference, with an oral and a poster presentation
- 2021 September: Hala Roumia leaves the project and Kadosa Sajdik (KS) joins the project as a research assistant
- 2021 October: TS gave a lecture on the online annual meeting of the COST Action "EsSENce" (CA19118)
- 2022 March: TS and PN give oral presentations on the 1st Regional Mini-Symposium of the AtomDeC consortium
- 2022 June: TS and assistant researcher KS participate in the 2nd CPBCI conference and have oral and poster presentations
- 2020 June: TS presents an overview of the research in the lab and regarding his collaboration with Prof Imre Dekany in the past 20 years, at the "2D Materials Science Days" of the University of Szeged
- 2022 June: The group members (TS, PN, KS) and the associated undergraduate students are the main organizers of the 18th Student Conference of the European Colloid and Interface Society (ESC-2022) held in Szeged. The students and KS present posters and PN holds a lecture at the conference. Details at: https://escconf2022.mke.org.hu/
- 2022 September: TS is at the 2022 European Colloid and Interface Science conference and provides the last oral dissemination of the project**. An extensive number of new collaboration possibilities have been discussed.**
- **2022 November: the project is terminated**

Szeged, 30th December, 2022

Tamas Szabo, PhD
Principal Investigator

National Research, Development and Innovation Office Hungary
PROGRAM FINANCED FROM THE NRDI FUND
MOMENTUM OF INNOVATION