GALLIC ACID LOADED GRAPHENE OXIDE MATERIAL AS AN EFFECTIVE ANTIBACTERIAL NANOCARRIER

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In this study, an antimicrobial nanodelivery system was developed using graphene oxide (GO) as nanocarrier loaded with gallic acid (GA) in different concentrations as bioactive agent (BA). The nanomaterials were characterized using FTIR, SEM, Raman and UV-Vis spectroscopy to determine the release behavior of BA. A burst delivery of BA was observed on the first day, followed by a sustained release for the next days. Evaluation of antimicrobial activity of samples was performed against S. aureus, E. coli and C. albicans. The nanomaterials demonstrated excellent antimicrobial efficacy with reduction rates above 90%, reducing also the microfungi populations up to 85%.

Keywords: graphene oxide, drug delivery systems, active substance, antibacterial activity

1. Introduction

Staphylococcus aureus and *Candida albicans* are the most common occurring bacterial and fungal agents that caused skin and soft tissue infections. These pathogens can cause hospital infections, representing an important threat to the global population [1, 2]. Because the use of synthetic antibiotics has increased, these species have developed resistance against synthetic anti-microbial compounds [3, 4]. Thus, there is an urgent need to develop novel drugs or novel drug delivery systems as anti-bacterial agents for the biomedical use.

In the last few years, drug delivery systems based on nanomaterials have been investigated and tested in the biomedical applications due to their efficient loading, controlled release, targeted delivery of drugs, improved drug solubility and therapeutic efficacy. Different functional nanocarriers such as graphene oxide, iron oxide magnetite nanoparticles, gold nanoparticles, polymeric nanoparticles, liposomes, micelles, etc., have been obtained and successfully applied in delivery

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of chemotherapy agents [5]. Graphene-based systems are a potential candidate for applications in biomedical areas, in drug delivery systems as antibacterial agents, due to their specific properties such as specific surface area, ability in hydrogen bond forming, π-π stacking, electrostatic and hydrophobic interactions with drugs, mechanical stiffness, thermal stability. Particularly, graphene oxide (GO) has a two-dimensional structure with unique features, such as high surface area, controlled oxygen-containing functional groups (carboxyl (COOH), hydroxyl (OH), epoxy groups etc.), good biocompatibility [6], being a promising material for biological applications such as delivering of antimicrobial drugs [7-9]. The chemical structure of GO is presented in Fig. 1.

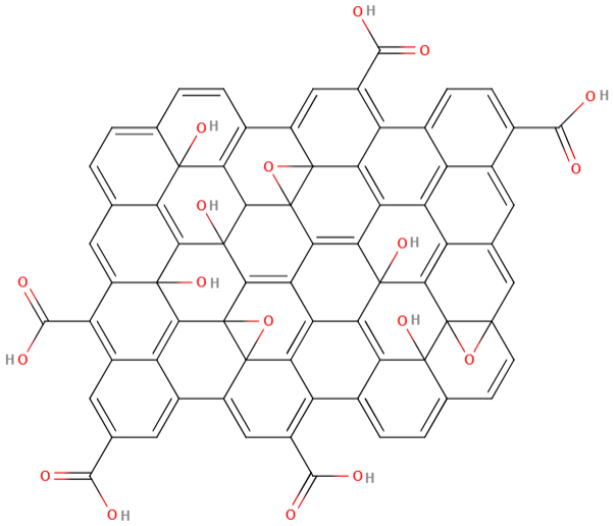


Fig. 1. Chemical structure of GO

In the past few years, it was found that GO has excellent ability to immobilize many substances, including metal nanoparticles, drugs, biomolecules and fluorescent molecules [10-12]. Elias *et al.* [13] investigated the antibacterial activity of gallic acid loaded graphene oxide (GOGA) nanoformulation against methicillin-resistant Staphylococcus aureus (MRSA). The results showed increased antibacterial activity of GA at lower concentration against MRSA (minimum inhibitory concentration (MIC): from 150 µg/mL to 100 µg/mL), demonstrating that GOGA has a promising potential as antibacterial agent against MRSA. Shamsi and collaborators [3] also investigated the antibacterial effect of GOGA against both MRSA and methicillin-sensitive SA (MSSA) at concentrations of 50-500 µg/mL. The obtained results highlighted anti-bacterial activity of GA against MRSA at lower concentration.

The present paper investigated the release profile and antimicrobial activity of nanostructured platforms based on graphene oxide as nanocarrier, loaded with gallic acid as biological active substance with antimicrobial activity.

The scope of this article is to highlight the potential development of GOGA as a promising anti-bacterial agent against multi-drug resistant bacteria, with application in healthcare system.

2. Materials and Methods

Graphene oxide, gallic acid (≥98%) (Merck), sodium chloride, sodium bicarbonate, magnesium chloride hexahydrate, hydrochloric acid 36,5-38%, sodium sulphate (Silal Trading), potassium chloride, dibasic potassium phosphate trihydrate, calcium chloride (Sigma Aldrich), tris-hydroximethyl aminomethane (Serva) were used in this paper.

2.1 Fabrication of GO-based materials

GO was obtained using modified Hummers method, presented in our previous literature report [14]. In order to obtain graphene oxide loaded with gallic acid (GOGA), different GA solutions were prepared using ethanol as solvent. In each solution was then added 0.2 g GO and the mixtures were stirred at room temperature until the solvent evaporated. The nanomaterials were then dried in a vacuum oven at 40°C for 24h. The final concentrations of the nanocomposites were: GOGA 40% and GOGA 20%.

To study the release profile of the loaded bioactive agent (BA) onto GO platforms, a phosphate buffer solution (FBS) with a pH of 7.4 was used. About 80 mg of each nanomaterial was added into 50 mL of FBS. From time to time (0.5, 1, 4, 6, 24, 48, 72 and 144 h respectively), aliquots were centrifuged for 10 min at 6000 rpm and 2 ml of sample solutions was taken and analysed by UV-Vis in order to evaluate the released amount of the BA. During this time, the solutions were kept in the oven at 37°C.

2.2 Evaluation of antimicrobial activity

Evaluation of antimicrobial activity of samples was performed by determining the logarithmic and percentage reduction, as well as the recovery rate of microorganisms. Recovery rate (%) serves as a very effective screening method when developing or evaluating new materials. When assessing the antimicrobial activity of new materials (with potential use as medical devices) this demonstration is critical in accurate determination of antimicrobial, disinfecting efficacy, bioburden, sterility or any test that requires determination of surviving microorganisms in a product containing antimicrobial properties. Failure to confirm adequate neutralization and recovery could result in under-reporting of surviving microorganisms. Three reference strains from the American Type Culture Collection (ATCC, Manassas, VA, USA) were used for testing (*Staphylococcus aureus* ATCC 6538, *Escherichia coli* ATCC 8739, *and Candida albicans* ATCC 10231). The microbial suspensions of 1.5×10^8 CFU/mL, obtained from fresh 15 to 18 hours cultures, developed on solid medium, were adjusted

according to McFarland standard 0.5, respectively McFarland 1 for microfungi, and then serially diluted to 10^{-5}. Samples were weighed and the inoculum volume was adjusted according to the mass of the samples. The samples were placed in contact with the microbial inoculum for 30 minutes, thoroughly spun on a vortex, and afterwards 5 decimal serial dilutions were carried out in order to determine the logarithmic and percentage reduction of the microbial populations. 10 µL in triplicate were inoculated in spot on Muller Hinton solid medium, respectively Sabouraud for microfungi. After 18-24 hours of incubation at 36 ± 2 °C the plates were read by counting the colonies.

The logarithmic reduction was calculated using the equation:

$$\text{Logarithmic reduction} = \lg \frac{A}{B} \quad (1)$$

A = no. of viable organisms before treatment; B = no. of viable organisms after treatment

The percentage reduction in microbial populations was calculated using the equation:

$$P = (1 - 10^{-L}) \times 100 \quad (2)$$

where P is the percentage reduction and L the logarithmic reduction

The determination of the recovery rate was carried out by performing 12 decimal serial dilutions from the inoculated samples with 10^{-5}, after 18 to 24 hours of incubation. The recovery factor was calculated using the equation:

$$RF = CFU\ positive\ control / CFU\ sample \quad (3)$$

The number of colonies obtained for the sample was compared with the ones obtained on the control plates. The number of colonies counted should not differ by more than a factor 2 (recovery rate 50% -200%).

2.3 Minimal inhibitory concentration (MIC) assay

The antimicrobial activity of the sample suspensions was assayed on Gram-negative (*Escherichia coli* ATCC 8739), Gram-positive (*Staphylococcus aureus* ATCC 6538) and microfungi (*Candida albicans* ATCC 10231) reference strains obtained from the American Type Culture Collection (ATCC, Manassas, VA, USA). Microbial suspensions of 1.5×10^{8} CFU/mL (0.5 McFarland density) obtained from 15 to 18 hours bacterial cultures developed on solid media were used. The samples were suspended in distilled water in order to prepare a stock solution of 200 mg/mL concentration. The quantitative assay of the antimicrobial activity was performed by the microdilution method in 96 multi-well plates. Decimal serial dilutions were performed. Culture positive controls (wells containing culture medium seeded with the microbial inoculum) were used. The plates were incubated for 24 hours at 37^{0}C, and the minimal inhibitory concentration (MIC) values were considered as the lowest concentration of the tested compound that inhibited the growth of the microbial overnight cultures, as

compared to the positive control. The samples absorbance was assessed at 620 nm using a microplate spectrophotometer.

3. Results and discussions

This report presents the characterization of the GO-based materials obtained by loading GO with natural substance, in particular gallic acid.

3.1 Fourier Transform Infrared Spectroscopy (FTIR)

FTIR spectroscopy was performed on the samples using a Nicolet iS50FT-IR spectrometer equipped with a DTGS detector.

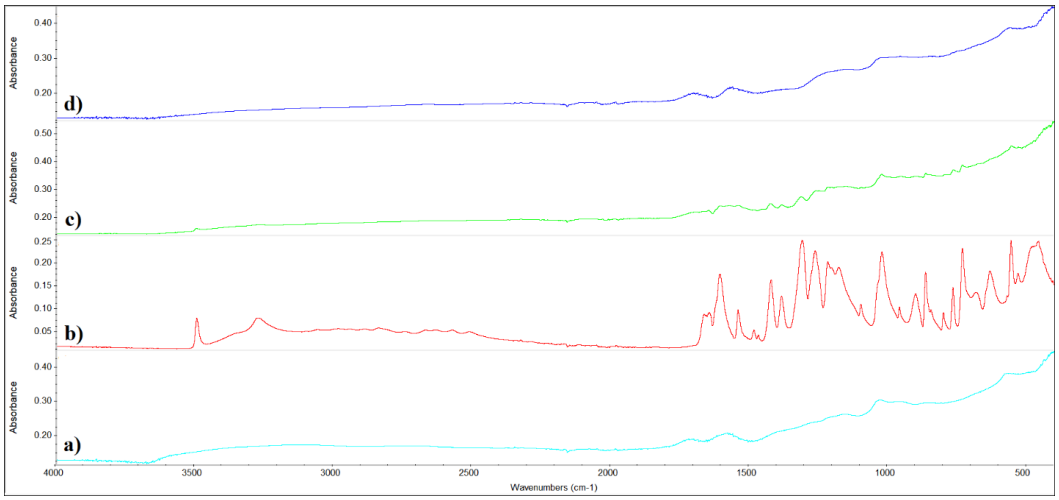


Fig. 2. FTIR spectra for (**a**) graphene oxide (GO), (**b**) gallic acid (GA), (**c**) GOGA 20% (gallic acid loaded onto GO nanocarrier), (**d**) GOGA 40%

As shown in Fig. 2(a),(b) FTIR spectra of GO and GA indicate the main characteristics peaks of GO and are similar to those found in previous literature reports [14-16]. After the loading of GA onto GO nanocarrier, GOGA does not show important changes in FTIR peaks, however the characteristic signals of both GO and GA are highlighted, suggesting the successful loading. The characteristic peaks of GO can be visualized but the relative intensity is lower because some of the bands are overlapped. Also, a visible shifting and broadening of the bands and an enhancement in the relative intensity of the peaks in GOGA 40% nanocomposite can be observed, due to the enhancement of GA concentration, which demonstrates the loading of GA onto GO [14, 17].

3.2 Scanning Electron Microscopy (SEM)

Scanning electron microscopy images were obtained using a high-resolution electron microscope equipped with a field emission electron source. The detailed surface morphology of GO loaded with biological active agents, was shown in Fig. 3. Higher magnification images were presented for a better

visualization. The morphology of GO sheets presented in our previous report [14] showed a very thin and lamellar structure on the macrolevel, but also the folded shape can be observed due to the high degree of functionalization [14, 18].

When GO was loaded with GA, no significant changes were observed, GOGA having an apparently smooth and irregular surface. Nevertheless, a slight increase in the degree of agglomeration of the particles can be observed because of the presence of the active compounds onto GO support, giving them a slightly irregular form [19, 20].

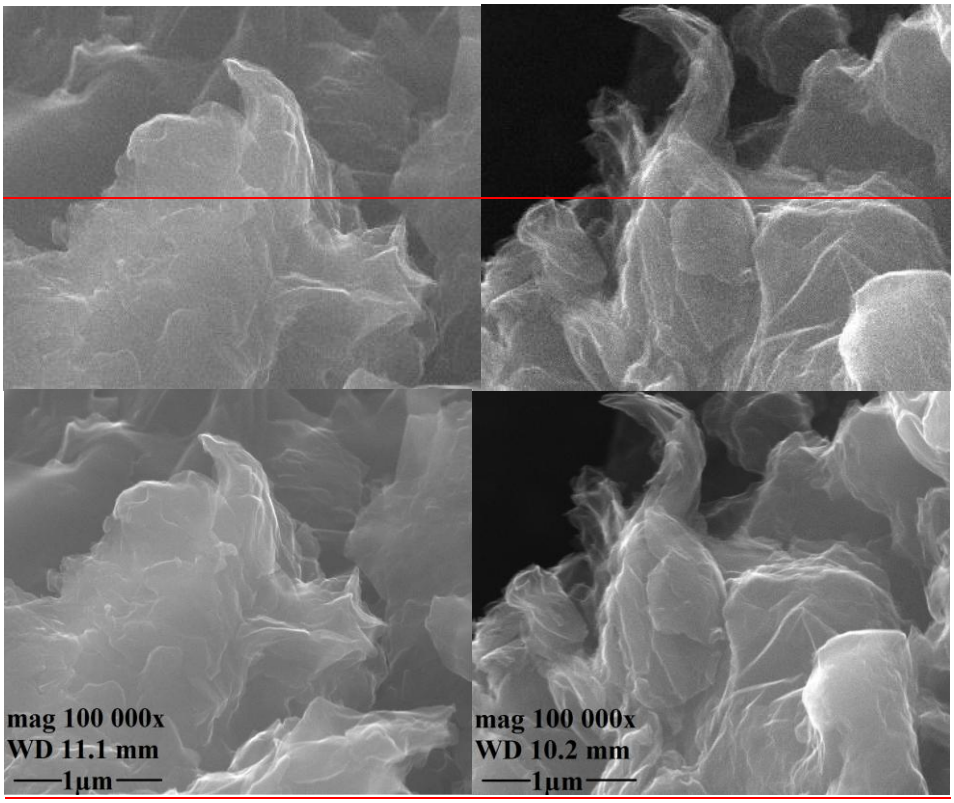


Fig. 3. SEM images for GOGA 40%-left, GOGA 20%-right

3.3 Raman spectroscopy

Raman spectroscopy was used to analyse the disorder and defects in the structure of GO and GO-based materials, using a Horiba equipment (with an excitation wavelength of 514 nm and a 50X objective (Fig. 4).

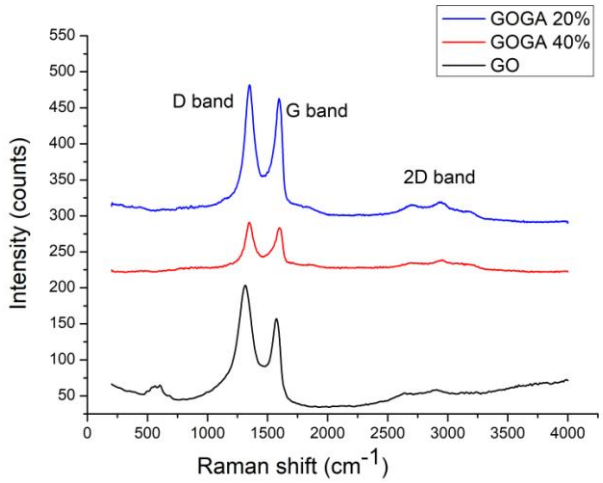


Fig. 4. Raman spectra of GOGA 40% and GOGA 20%

Fig. 4 shows the Ramana spectra for GO support loaded with BA, gallic acid. The disorder in GO can be determined by measuring the intensity ratio corresponding to D and G bands [21]. The Raman spectrum for GO is similar to the spectra presented in other literature reports [22, 23]. The peaks around 1350 and 1600 cm^{-1} are representative for D and G bands, in GO samples. After addition of GA into the GO nanocarrier, structural changes occurred: the peak intensity of D and G bands (corresponding to sp_3 and sp_2 carbon forms) is decreasing due to the enhancement of active compound concentration, confirming their adsorption on the surface of GO [24].

The 2D band can determine the number of layers from GO nanomaterials. If 2D band occurs as a single peak, is considered to be specific for single layer GO. An increase in the number of layers increases the number of 2D peaks and also reduces the intensities of the 2D peaks [16]. The 2D band occurs in the range of 2500 and 3300 cm^{-1}. The ratio I_{2D}/I_G for all samples is between 1.84 and 1.85 for all samples, these results indicating that GO materials are in the form of multilayers [25].

3.4 Release Behaviour of Active Compounds

A UV-Vis JASCO (Easton, PA, USA) V560 spectrophotometer was used to measure the amount of active substances released into the FBS. Absorbance values were measured at 266 nm for GOGA samples. Standard curve for GA has been used. The release profile of GA from GOGA 40% and GOGA 20% (Fig. 5) was significantly different using the same conditions, the maximum release ratio reaching 12.6% in the first 2h for GOGA 40% and 8.9% at 72 h for GOGA 20%. GOGA 20% nanocarrier showed a sustained/gradual and slow release, compared to the initial burst release of GA from GOGA 40%. The initial burst release for GOGA 40% observed in the first 2h can be attributed to the hydrogen-bonding

interactions between GO structure and GA. Due to the large amount of GA loaded onto GO nanocarrier, GA can remain unattached onto the surface of GO support leading to a fast release of the drug [3, 26, 27]. GA from GOGA 20% did not released 100% due to ion-exchange reaction mechanism, but the drug was removed continuously. The obtained results demonstrated good potential for GOGA nanocomposite as drug delivery system.

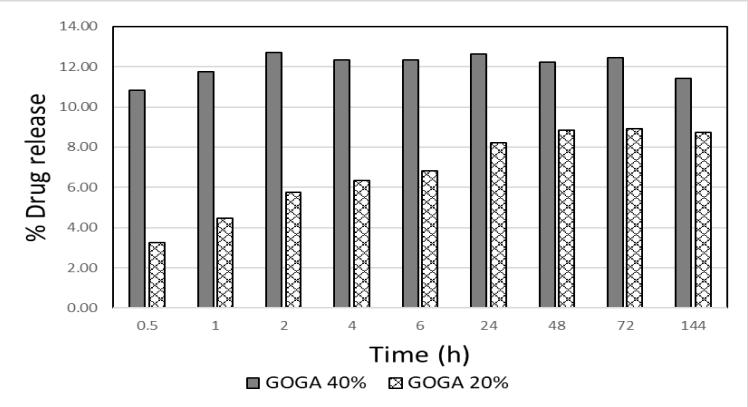


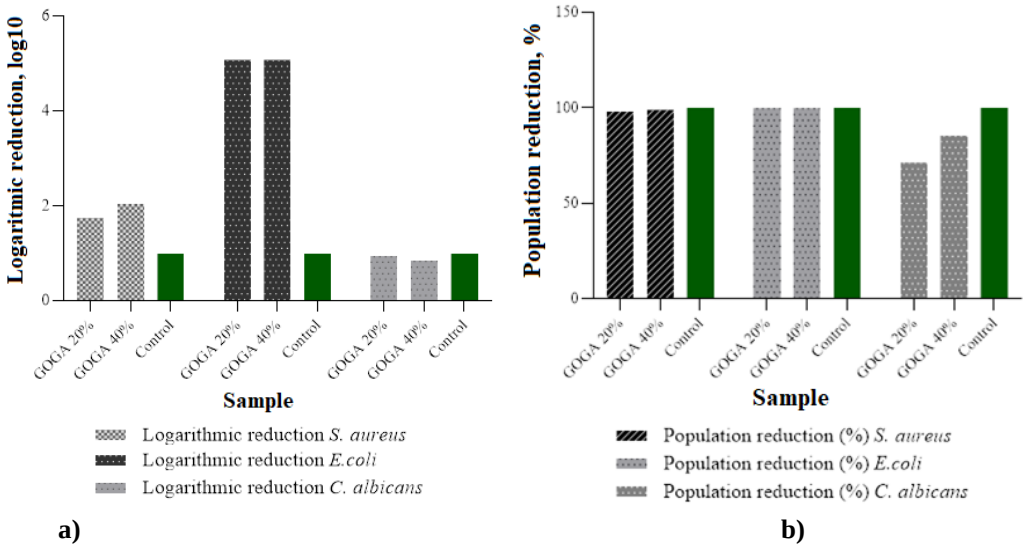
Fig. 5. The release profile for GO-based materials

3.5 Evaluation of antimicrobial activity and MIC assay

Samples represented by gallic acid (20 % and 40 %) loaded on graphene oxide as support were evaluated in the presence of Gram-positive, Gram-negative and microfungi reference strains. Logarithmic factor 1, which represents a 90% efficiency in bacterial reduction, was considered as a control.

The results on logarithmic reduction presented in Fig. 8 show a 5log reduction for the *E. coli* strain, samples being most effective on this Gram-negative strain. On *S. aureus* the logarithmic reduction was 1.74 and 2.04, such results indicating excellent antimicrobial efficiency. Results obtained for the *C. albicans* strain indicate a 0.54 and 0.84 log reduction, relatively modest compared with values obtained on Gram strains.

In correlation with the logarithmic reduction, the population reduction (%) results for all strains tested are presented in Fig. 6. A 100 % reduction was considered as control. On *S. aureus* and *E. coli* strains all samples manifested excellent antimicrobial activity, with reduction rates above 90 %. As for the *C. albicans* strain, reduction percentages were 71 % for sample GOGA 20% and 85 % for sample GOGA 40%.



a) **b)**

Fig. 6. a) Logarithmic reduction and b) Population reduction of *S. aureus* ATCC, *E. coli* ATCC *and C. albicans* ATCC strains by samples GOGA 40%, GOGA 20%

As the European Pharmacopoeia recommends for antimicrobial substances, the recovery rate must not differ with more than a 2 factor (50% - 200% recovery). Therefore, in this interval, sample GOGA 40% falls within this range for *E. coli* and *C. albicans* strains.

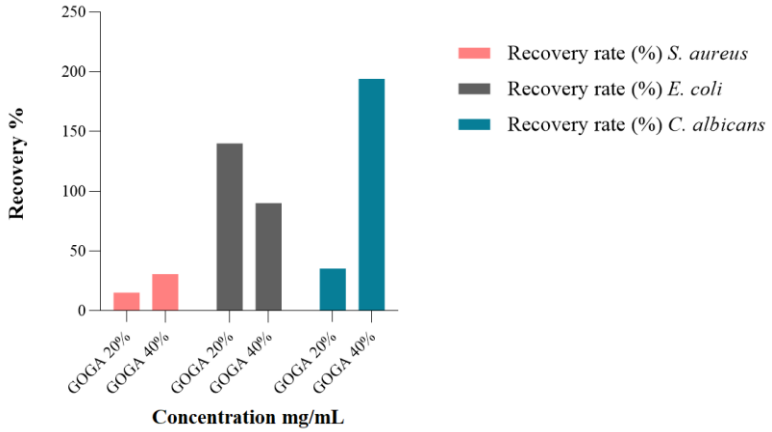


Fig. 7. Recovery rate (%) of *S. aureus* ATCC, *E. coli* ATCC *and C. albicans* ATCC strains by samples GOGA 40%, GOGA 20%

Sample GOGA 20% falls within the indicated range only for the *S. aureus* strain, therefore confirming its antimicrobial efficiency and correlates as a trend with the logarithmic and percentage reduction results. The MIC value was assessed by the microdilution method, against *S. aureus*, *E. coli*, and *C. albicans* strains, all being considered representative microorganisms for their group and

significant pathogens associated with various post-op infections. For the MIC assay were tested the following concentrations: 200 mg/mL, 100 mg/mL, 25 mg/mL, 3.125 mg/mL and 0.195 mg/mL. The results obtained and presented in Fig. 8 indicate that for *E. coli* and *C. albicans* strains the MIC values were obtained at 0.195 mg/mL for all sample tested. As for the *S. aureus* strain, the MIC was obtained at a higher concentration (3.125, mg/mL).

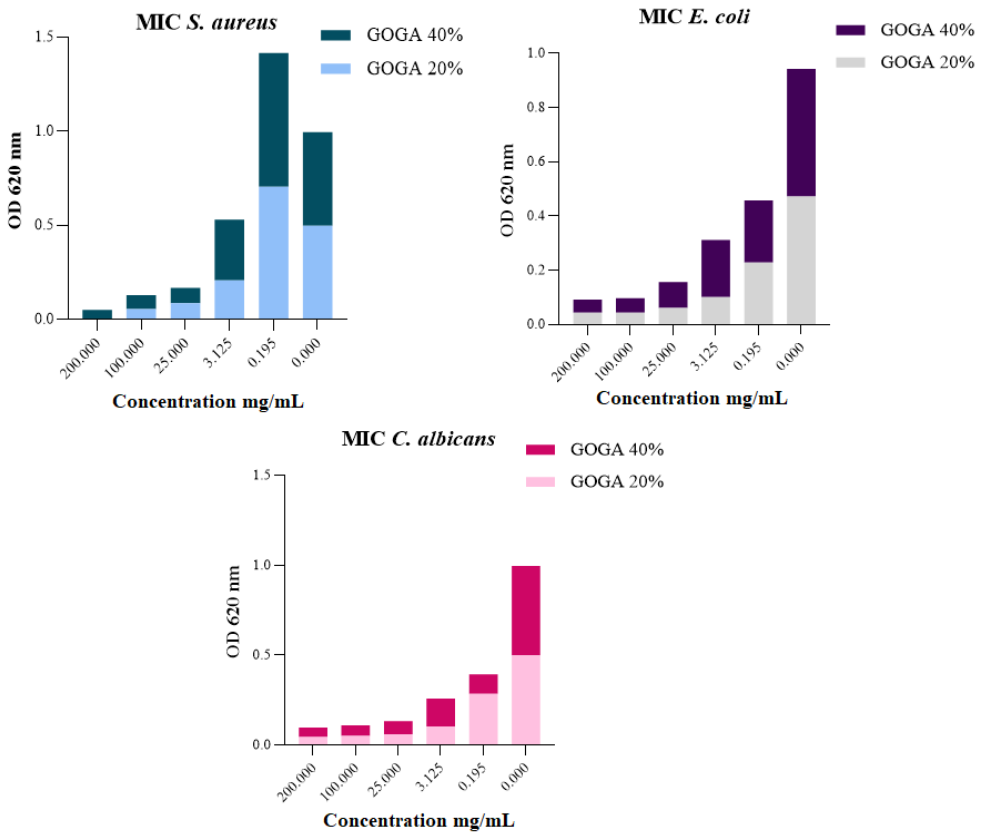


Fig. 8. Minimal inhibitory concentrations of samples GOGA 40%, GOGA 20%

When evaluated as a trend, the obtained results correlate properly and indicate excellent antimicrobial efficacy on *S. aureus* and *E. coli* strains, especially for sample GOGA 40%. As for the *Candida* strain, the obtained effect fits into the fungistatic action, reducing the microfungi populations up to 85%.

4. Conclusions

The scope of this paper was to obtain antibacterial GOGA nanomaterials as drug delivery systems, using different concentrations of GA. The obtained nanocomposites demonstrated excellent antimicrobial efficacy on both *S. aureus* and *E. coli* strains with reduction rates above 90 % and fungistatic action reducing

the *Candida* populations up to 85%. The release behavior results demonstrated sustained release for GA from GOGA 20% over longer period of time, thus increasing the viability of drug showing better therapeutic efficacy. Therefore, the designed platforms demonstrated good potential for GOGA as drug delivery system with antimicrobial activity. These results have encouraged this research towards the next level by carrying *in vivo* studies to demonstrate the safe use of these platforms as antimicrobial agents for different biomedical applications.

Acknowledgments

The work has been funded by the Operational Programme Human Capital of the Ministry of European Funds through the Financial Agreement 51668/09.07.2019, SMIS code 124705, Core Program 16N/08.02.2019

R E F E R E N C E S

[1] H. Carolus, K. Van Dyck, P. Van Dijck, Candida albicans and Staphylococcus Species: A Threatening Twosome, Front Microbiol, 10, 2019, 2162.

[2] A. Mekonnen, B. Yitayew, A. Tesema, S. Taddese, In vitro antimicrobial activity of essential oil of Thymus schimperi, Matricaria chamomilla, Eucalyptus globulus and Rosmarinus officinalis, International Journal of Microbiology 2016, 2016, 1-8.

[3] S. Shamsi, N. Elias, S.N.E. Sarchio, F.M. Yasin, Gallic Acid Loaded Graphene Oxide Based Nanoformulation (GAGO) as Potential Anti-bacterial Agent against Staphylococcus aureus, in: A. Dalton, B.T. Goh (Eds.) 7th Asian Conference on Colloid and Interface Science, Materials Today:Proceeding, Kuala Lumpur, MALAYSIA, 2017, S160-S165.

[4] F. Bugli, M. Cacaci, V. Palmieri, R. Di Santo, R. Torelli, G. Ciasca, M. Di Vito, A. Vitali, C. Conti, M. Sanguinetti, M. De Spirito, M. Papi, Curcumin-loaded graphene oxide flakes as an effective antibacterial system against methicillin-resistant Staphylococcus aureus, Interface Focus, 8(3), 2018, 20170059.

[5] D.R. Vogus, V. Krishnan, S. Mitragotri, A review on engineering polymer drug conjugates to improve combination chemotherapy, Curr Opin Colloid In, 31, 2017, 75-85.

[6] E. Cojocaru, A.M. Onas, H. Iovu, Carboxilated graphene oxide integrated chitosan composite scaffolds as encouraging materials for tissue engineering, UPB Sci. Bull. Series B 82, 2020, 15-28.

[7] A. Anand, B. Unnikrishnan, S.-C. Wei, C.P. Chou, L.-Z. Zhang, C.-C. Huang, Graphene oxide and carbon dots as broad-spectrum antimicrobial agents – a minireview, Nanoscale Horizons, 4, 2019, 117-137.

[8] P. Kumar, P. Huo, R. Zhang, B. Liu, Antibacterial Properties of Graphene-Based Nanomaterials, Nanomaterials (Basel), 9, 2019, 737.

[9] M.P. Romero, V.S. Marangoni, C.G. de Faria, I.S. Leite, C. de Carvalho Castro e Silva, C.M. Maroneze, M.A. Pereira-da-Silva, V.S. Bagnato, N.M. Inada, Graphene Oxide Mediated Broad-Spectrum Antibacterial Based on Bimodal Action of Photodynamic and Photothermal Effects, Front Microbiol, 10, 2020, 2995.

[10] D.M.J. Seema, B. Saifullah, M. Selvanayagam, S. Gothai, M.Z. Hussein, S.K. Subbiah, N.M. Esa, P. Arulselvan, Designing of the Anticancer Nanocomposite with Sustained Release Properties by Using Graphene Oxide Nanocarrier with Phenethyl Isothiocyanate as Anticancer Agent, Pharmaceutics, 10(3), 2018, 109.

[11] A. Croitoru, O. Oprea, A. Nicoara, R. Trusca, M. Radu, I. Neacsu, D. Ficai, A. Ficai, E. Andronescu, Multifunctional Platforms Based on Graphene Oxide and Natural Products, Medicina-Lithuania, 55, 2019, 230.

[12] A.M. Croitoru, A. Ficai, D. Ficai, R. Trusca, G. Dolete, E. Andronescu, S.C. Turculet, Chitosan/Graphene Oxide Nanocomposite Membranes as Adsorbents with Applications in Water Purification, Materials, 13(7), 2020, 1687.
[13] N. Elias, S.N.E. Sarchio, F. Md Yasin, S. Abd Gani, S. Shamsi, Antibacterial effects of gallic acid loaded graphene oxide (GAGO) against methicillin-resistant Staphylococcus aureus (MRSA), Frontiers in Pharmacology, 9, 2018, DOI:10.3389/conf.fphar.2018.3363.00108.
[14] A. Croitoru, O. Oprea, A. Nicoara, R. Trusca, M. Radu, I. Neacsu, D. Ficai, A. Ficai, E. Andronescu, Multifunctional Platforms Based on Graphene Oxide and Natural Products, Medicina-Lithuania, 55, 2019.
[15] W. Han, W.Y. Niu, B. Sun, G.C. Shi, X.Q. Cui, Biofabrication of polyphenols stabilized reduced graphene oxide and its anti-tuberculosis activity, J Photoch Photobio B, 165, 2016, 305-309.
[16] E. Aliyev, V. Filiz, M.M. Khan, Y.J. Lee, C. Abetz, V. Abetz, Structural Characterization of Graphene Oxide: Surface Functional Groups and Fractionated Oxidative Debris, Nanomaterials-Basel, 9(8), 2019, 1880.
[17] D. Dorniani, B. Saifullah, F. Barahuie, P. Arulselvan, M.Z. Bin Hussein, S. Fakurazi, L.J. Twyman, Graphene Oxide-Gallic Acid Nanodelivery System for Cancer Therapy, Nanoscale Res Lett, 11, 2016, 491.
[18] N.I. Zaaba, K.L. Foo, U. Hashim, S.J. Tan, W.W. Liu, C.H. Voon, Synthesis of Graphene Oxide using Modified Hummers Method: Solvent Influence, Procedia Engineering, 184, 2017, 469-477.
[19] Q. Zhang, X. Huang, Y.Q. Pu, Y.X. Yi, T. Zhang, B. Wang, pH-sensitive and biocompatible quercetin-loaded GO-PEA-HA carrier improved antitumour efficiency and specificity, Artif Cell Nanomed B, 46, 2018, S28-S37.
[20] J.P. Long, S.X. Li, J.M. Liang, Z.G. Wang, B. Liang, Preparation and characterization of graphene oxide and it application as a reinforcement in polypropylene composites, Polym Composite, 40, 2019, 723-729.
[21] Z.Y. Jiang, H.L. Jin, S. Sun, C.Q. Chen, J. Zhang, Z.F. Guo, X.Y. Liu, Effects of gallic acid biofabricated rGO nanosheets combined with radiofrequency radiation for the treatment of renal cell carcinoma, Mat Sci Eng C-Mater, 93, 2018, 846-852.
[22] E.A. Eremina, A.V. Kaplin, A.A. Eliseev, A.V. Sidorov, S.S. Radzhabzoda, A.V. Grigor'eva, E.A. Gudilin, Multifunctional Composites Based on Graphite Oxide, Doxorubicin, and Magnetic Nanoparticles for Targeted Drug Delivery, Nanotechnol Russ, 13, 2018, 152-160.
[23] F. Kordi, A.K. Zak, M. Darroudi, M.H. Saedabadi, Synthesis and characterizations of Ag-decorated graphene oxide nanosheets and their cytotoxicity studies, Chem Pap, 73, 2019, 1945-1952.
[24] Y.G. Yuan, Y.H. Wang, H.H. Xing, S. Gurunathan, Quercetin-mediated synthesis of graphene oxide-silver nanoparticle nanocomposites: a suitable alternative nanotherapy for neuroblastoma, Int J Nanomed, 12, 2017, 5819-5839.
[25] R. Muzyka, S. Drewniak, T. Pustelny, M. Chrubasik, G. Gryglewicz, Characterization of Graphite Oxide and Reduced Graphene Oxide Obtained from Different Graphite Precursors and Oxidized by Different Methods Using Raman Spectroscopy, Materials, 11(17), 2018, 1050.
[26] F. Luzi, E. Pannucci, L. Santi, J.M. Kenny, L. Torre, R. Bernini, D. Puglia, Gallic Acid and Quercetin as Intelligent and Active Ingredients in Poly(vinyl alcohol) Films for Food Packaging, Polymers-Basel, 11(12), 2019, 1999.
[27] M. Islami, A. Zarrabi, S. Tada, M. Kawamoto, T. Isoshima, Y. Ito, Controlled quercetin release from high-capacity-loading hyperbranched polyglycerol-functionalized graphene oxide, Int J Nanomed, 13, 2018, 6059-6071.