ANALYSIS OF DOXORUBICIN/GRAPHENE QUANTUM DOT COMPLEX FORMATION USING BLACK SILICON-BASED SERS SUBSTRATE

Lena Golubewa

Center for Physical Sciences and Technology, Department of Molecular Compound Physics
Saulėtekio av. 3, LT-10257 Vilnius, email: lena.golubewa@ftmc.lt

Doxorubicin (DOX) is one of the chemotherapeutic agents used in the treatment of cancer. Its disadvantage is poor selectivity for target cells and uncontrolled distribution throughout the body, which causes serious side effects. In this regard, the development of non-toxic carriers of doxorubicin for targeted delivery of the drug to the tumor tissue is an actual task. The carrier should have high tissue permeability, loading capacity and be able to release the drug in controllable way. Ideally, the carrier should be a fluorescent particle allowing image-guided drug delivery to the target tissue. Boron nitride quantum dots (BNQDs) have been already demonstrated to act as such a fluorescent shuttle for DOX molecules. However, BNQDs are not that efficient because of their UV-blue luminescence overlaping significantly with tissue autofluorescence.

Green fluorescent graphene quantum dots (GQDs) are likely to become carriers appropriate for image-guided DOX delivery to the target tissue: (i) GQDs are flakes of a few layers of graphene suitable for π-stacking mechanism of complex formation with large variety of analyties likely including DOX; (ii) GQDs are biocompatible, non-cytogenic or cytotoxic; (iii) five nm sized GQDs exhibit bright green fluorescence [1].

To produce reliable protocol for application of GQDs as secure and firm carrier for DOX, investigation of DOX-GQD complexes formation was performed. Suspension of DOX and GQDs of different concentrations (10^{-6}-10^{-4} M and 25-50 μg/mL, respectively) were analyzed using steady-state and time-resolved fluorescence spectroscopies. Concentration and time-dependent decrease in characteristic lifetimes was demonstrated. To reveal the mechanism of DOX-GQD complexes formation, photoluminescence excitation/emission (PLE) data maps of DOX-GQD complexes of different concentrations and at various time points were obtained and obvious differences between the PLE characteristics of the complexes and those of the GQDs and DOX were shown.

The concentrations of DOX and GQDs are in the ranges of 10^{-6}-10^{-5} M and 25-50 μg/mL, respectively, which makes conventional Raman spectroscopy approach inappropriate. In the present study we applied black silicon sputtered with gold (bSi/Au) as a substrate for surface enhanced Raman spectroscopic (SERS) investigation of DOX, GQDs and DOX-GQDs complexes. BSi is a specifically etched silicon wafer with high curvature cone-like structures of micrometers height, which increase absorbance and suppress reflectivity. After being sputtered with gold, these structures serve as active sites for electromagnetic field enhancement (hot spots) and induce Raman signal enhancement up to 10^8 times (for the bSi/Au used in this study [2]). Unlike Raman spectroscopy which failed in getting any valuable spectra of low-concentration solutions, SERS spectra were obtained for all investigated complexes and complex-forming agents.

SERS spectra allowed revealing the complex structure of functionalized GQD, i.e., green fluorescent GQDs (900712, Sigma Aldrich) strongly passivated with oxygen-containing groups, which are involved in additional DOX molecule orientation on the graphene island. Based on the band shifts in the spectra, as well as on the change in the relative intensity of the doxorubicin and GQD bands, the main functional groups engaged in the formation of the complex were identified.

The obtained data were proved by further DFT calculations, which were carried out both for individual compounds and for their conjugates in vacuum and in an aqueous medium, imitating the cytoplasm of living cells. SERS spectral data on high enrichment of GQDs with oxygen-containing groups became the basis for GQD model modification. Calculations were performed for GQD, consisting of 114 carbon atoms, and containing 10 hydroxyl groups (GQD_C114H20(OH)10). A comparative analysis of the electronic structure of DOX and the GQD–DOX complex revealed the decrease in the $\Delta E = E_{HOMO} - E_{LUMO}$ difference between the energies of the lowest non-occupied and highest occupied molecular orbitals, allowing to assume the formation of a stable complexes. Moreover, the dipole moment of GQD obtained after geometry optimization significantly increased when complex with DOX is formed.

To summarize, in the present study concentration and time-dependency of DOX-GQD complexes formation was demonstrated. SERS spectroscopic analysis allowed to reveal the main functional groups and GQD structures responsible for the stable complexation of DOX molecules with GQDs and provided sufficient data on the functionalized GQD structure, which were further used for DFT simulations of DOX-GQD complexes. The obtained results will be used to develop methods of controllable release of doxorubicin in the target tissue.

References

1. Golubewa, L. et al. Nanotechnology **33**, 15 (2022)
2. Golubewa, L. et al. ACS Appl. Mater. Interfaces **12**, 50971–50984 (2020).