Few-layer graphene dispersion in liposomes with antibacterial activity

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In recent years graphene and its derivatives, due to their unique physicochemical properties, have aroused interest in many research fields. As a matter of fact, applications in electronic [1] and photonic devices [2], clean energy [3], energy storage [4] and sensors [5] have been well demonstrated. In addition, graphene-based materials appear as promising scaffolds in biomedicine [6-8]. For application in biomedicine one of the most important and fundamental goal to be achieved is to make graphene soluble in water. Up to now, almost all the studies on graphene in the biomedical field have been focused on the production and characterization of hydrophilic graphene oxide (GO). The functionalization of graphene to get GO has revealed undisputed advantages, but it involves the breakdown of the continuous honeycomb backbone of pristine (non-functionalized) graphene compromising several of the peculiar properties of the original material.

In order to preserve graphene structural integrity and to use an absolutely green process of exfoliation and functionalization, inspired by Titov's molecular dynamics simulation [9] that showed the theoretical insertion of a *"graphene sheet in the hydrophobic interior of biological membranes"*, in the present study we succeeded to realize a facile and prompt exfoliation protocol for graphite in aqueous solution.

We obtained a liposomal formulation in which graphene is sandwiched between phospholipid alkyl chains by simply sonicating graphite into POPC large unilamellar vesicle aqueous solution for 2 h. The obtained results were validated and quantified by the use of different methods of investigation such as: UV-Vis spectrophotometry to quantify the exfoliated graphene (yield~15%) and the liposomal entrapment efficiency (E.I.~2%), Raman spectroscopy to demonstrate the presence of non-oxidized double layer graphene as well as amphiphilic phospholipid molecules organized in bilayers in the samples, TEM to point out that sonication allows the formation of nanometric double-layer graphene sheets embedded into liposomes, DLS and ζ–potential analyses to evaluate the suspension stability over time and homogeneity of the dispersion.

The as-prepared graphene aqueous dispersion is stable for days and demonstrates significant antibacterial activity against both Gram-positive (Staphylococcus aureus) and Gram-negative (Escherichia coli) strains. In particular, it almost completely inhibits the microbial growth of Gram-positive bacteria whereas Gram-negative bacterial growth is reduced to a mere three-fold.

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