INDIAN INSTITUTE OF TECHNOLOGY GUWAHATI
SHORT ABSTRACT OF THESIS

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Thesis Title: **Structural, Functional and Morphological Modifications of Graphene Oxide-based Nanocarriers for Anticancer Drug Delivery**

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SHORT ABSTRACT

Chemotherapy has continued to be the most frequently utilized therapy for cancer treatment. Despite the abundance of chemical chemotherapeutic agents and anticancer medications, their unregulated administration and inability to distinguish between rapidly proliferating healthy cells and cancer cells lead to adverse impacts on cancer therapeutics. Moreover, due to the diversity of cancer cells, the emergence of drug resistance, and the undesirable effects induced by high and/or repetitive drug doses, the administration of a solitary chemotherapeutic agent frequently fails to accomplish a comprehensive cancer regression. The major challenges related to effective and sustainable consumption of chemotherapeutic agents can be subsided with targeted drug delivery systems (DDS) directed by the advanced nanomaterials (nanocarriers), natural chemotherapeutic agents such as gallic acid (GA), caffeic acid (CA), targeting ligands such as folic acid (FA) and employing co-delivery systems for chemotherapeutic agents. Previous studies related to anticancer DDS have demonstrated the therapeutic potential of graphene-based nanocarriers due to their distinct surface characteristics.

Graphene oxide (GO), a 2D hydrophilic derivative of graphene, is enriched with oxygenated functional groups and delivers significant advantages for improved drug loading and functionalization with targeting ligands and biocompatible biopolymers. Despite the several

properties and applications of GO, it owns some disadvantages, like agglomeration or overlapping of GO sheets, larger lateral dimensions, less porous structure and the lower stability of GO suspension in an organic solvent. These limitations collectively affect the loading and release characteristics of GO-based nanocarriers along with their toxicological aspects. In line with the limitations of GO for drug delivery-related applications, this thesis work is focused on the modifications of surface characteristics of GO-based nanocarriers for anticancer DDS. These nanocarriers were structurally, functionally, and morphologically modified to improve their colloidal stability, targetability, cytocompatibility, loading, and release characteristics for conventional as well as natural chemotherapeutics agents.

Initially, this work elucidates a facile method for the structural and morphological modification of GO nanosheets into spirally wounded 1D graphene oxide nanoscrolls (GONS) of tunable dimensions by low frequency (20 kHz) ultrasonication. The GO was synthesized by modified Hummers' method using low-cost synthetic graphite as a base material. Later, GO suspension (0.05 gL^{-1}) was made using methanol as solvent and subjected to different ultrasonication conditions. GO sheets were found to curl themselves into nanoscrolls by overcoming the energy barrier for scrolling with the help of bubble cavitation energy provided by ultrasonication. The effect of ultrasonication power (100 - 150 W) for irradiation time (0.5 -3 h) over the GONS dimensions was investigated. The spiral wounded GONS structure was visualized through electron microscopy. Thin-film X-ray diffraction, Raman, X-ray photoelectron, and FTIR spectroscopic analysis were also conducted to endorse GONS formation. Factors affecting GONS formation, such as sonication power and solvent selection, were studied as scrolling of GO sheets is strongly dependent on sonication parameters and solvent characteristics. It was found that the average length of obtained GONS decreases from 1311 nm to 1011 nm and 1165 nm to 516 nm for 100 W and 150 W sonication power, respectively, with an increase in irradiation time from 0.5 h to 3 h during ultrasonication. It was expected that the GONS with improved surface characteristics and morphology might support its applications in anticancer DDS.

Later, the obtained spirally wounded GONS are employed as nanocarriers for their applications in anticancer DDS. GONS nanocarriers were loaded with GA at different compositions, temperatures and pH of the media. In addition, an *in-vitro* study was conducted on drug loading and release behavior at physiological pH conditions to understand the GA loading mechanism, thermodynamics,

and release kinetics. The presented structural and morphological modification of GO into 1D GONS was favorable for a significant improvement in the loading and release behavior of GA for cancer therapeutics with a maximum loading efficiency of $29.82 \pm 0.8\%$ at a 1.25 weight ratio of GA and 73.78 ± 2.21 % GA release in 5 days. The cytotoxicity studies of GONS-GA nanocomposites demonstrated higher toxicity for lung cancer cell lines (A549) without showing perceptible cytotoxicity for normal cell lines (HEK293), which advocates GONS as a suitable nanocarrier for the DDS.

Furthermore, in line with the targeted co-delivery principle for chemotherapeutic agents, the fabricated nanoscrolls were firstly functionalized with chitosan (CS) biopolymer and FA to further enhance their biocompatibility and target-specific delivery of anticancer drugs. The resultant GONS-CS-FA (GCF) nanocarriers were loaded with DOX and CA at different weight proportions with respect to nanocarrier and drug composition. The optimum loading efficiency of 51.14 ± 1.47 % (DOX) and 49.70 ± 1.19 % (CA) was observed for a drug: GCF ratio of 2.5 with drug composition of 1:1. It was observed that with an increase in drug composition DOX: CA, in contrast to CA the loading efficiency of DOX was enhanced. Additionally, to explore the release behavior of the GCF nanocarrier, an *in-vitro* release study was performed for GCF-DOX-CA nanocomposite under pH conditions mimicking both physiological and cancer micro-environments. At pH 5, ~ 83 and 75 % DOX and CA were released from the GCF-DOX-CA nanocomposite, respectively, whereas ~71 and 61 % release was observed at pH 7.4 over the course of 7 days, suggesting a higher and targeted drug release in the cancer microenvironment. The *in-vitro* cytotoxicity evaluations of GCF-DOX-CA nanocomposite demonstrated significant cytocompatibility for normal cells (HEK293) and selective apoptosis for cancer cell lines (A549).

In addition, a sustainable, facile, and one-pot synthesis approach is adapted for the preparation of GO-impregnated Fe-based metal-organic frameworks [(GO/MIL-100(Fe)) (Gw-MF)] as doxorubicin (DOX) nanocarriers via the HF free route. The DOX loading efficiency of these nanocarriers was studied for Gw-MF at a fixed drug concentration and with varying DOX with respect to the carrier (w/w). Amongst various Gw-MF nanocarriers, G0.5-MF has shown a superior loading efficiency of 29.91 ± 1.49 % for a 1 w/w ratio of DOX. The DOX-released pattern of the G0.5-MF nanocarrier was more controlled in nature, which may improve the therapeutic efficacy. The release behavior of G0.5-MF was further studied using kinetic modeling. *In-vitro* cytotoxicity studies of G0.5-MF

showed higher toxicity to cancer cells (A549) without showing noticeable toxicity for normal cells (HEK293). A similar approach is adopted for synthesizing CA nanocarriers using β-cyclodextrin conjugated graphene oxide (β-CD-GO)-functionalized Fe-based metal-organic frameworks [(β-CD-GO@MIL-100(Fe)) (BGw-MF)]. The structural, functional, and morphological attributes of obtained nanocarriers were inspected through respective characterization methods. Furthermore, the CA loading efficiency (LE) was assessed for various BGw-MF nanocarriers, with fixed drug concentration and variable CA masses (w/w) with respect to the carrier and pH of the media. The BG0.5-MF nanocarrier presented the highest LE for CA, reaching 56.44 ± 2.25% for a 2 w/w ratio of CA in a basic environment (pH 9). Moreover, the CA release pattern of BG0.5-MF-CA was controlled. The release characteristics of BG0.5-MF were subsequently investigated using a kinetic study. *In-vitro* cytotoxicity tests of BG0.5-MF-CA revealed significant toxicity to lung cancer cells (A549) deprived of inducing evident toxicity to normal cells (HEK293). Overall, a room temperature and sustainable synthesis approach of GO@MOF-based nanocarriers has emphasized their potential for higher drug loading capacity, improved release characteristics, suitable colloidal stability, and cytotoxicity performance towards cancer cells in the field of DDS.