4.1 Introduction

The allotropes of carbon nanomaterials (carbon nanotubes, graphene) are unique and most promising candidates for material chemists in last decade. Due to their nanoscale diameter and high aspect ratio, a little amount of these nanomaterials is able to produce a dramatic improvement in the properties of these materials. Although carbon nanotubes (CNTs) and graphene exhibit extraordinary properties, their reported commercialization is still limited due to their bundle and layer forming behavior. Functionalization of CNT and graphene is essential for achieving their outstanding mechanical, electrical and biological functions and enhancing their dispersion in polymer matrix. Idea of modifying different materials and their surfaces at nano scale has been satisfactorily exploited in recent decades, especially by nano chemists and technologist. Synthesis of carbon nanomaterials (CNMs) and modifications of their surfaces undoubtedly provides an opportunity to bolster scientific efforts in order to create a more skilled and resourceful world community capable of confronting its challenges. Functionalized carbon nanomaterials have unlocked a wide spectrum of applications in various fields. Importance of modified nano surface is well reflected in scientific work of last decade. Among carbon nanomaterials, carbon nanotubes (CNTs) and graphene have many superior properties such as low-weight, very high aspect ratio, high electrical conductivity, extraordinary mechanical, optical, and thermal properties (1-3). Carbon nanotubes (CNTs) and graphene have built broad interest in most areas of science and engineering because of their extraordinary physical, mechanical, thermal and optical properties (4). Applications of carbon nanomaterials in various fields are assisted by functionalization on their surfaces. The nonpareil physiochemical features of these functionalized nanomaterials have been exploited by various workers for energy applications (4), in the treatment of cancer (5-8), antiviral drug development (9), drug transportation in a biological system (10-15), biotechnological aspects (16,17) and modified aerospace materials (18,19). In addition, some theoretical efforts have also been made to analyze and optimize functionalization for various purposes (20, 21). Non-functionalized nanomaterials of carbon possess some inextricable drawbacks in their pristine form, such as tendency to form stable aggregates or bundles by very strong intermolecular interactions such as van der Waals forces, dipole-dipole interactions, etc. The aggregation of nanomaterials results in unwanted changes and
hampering of various physiochemical properties, especially their mechanical and electrical properties. The best nanomaterial support system could only be achieved by stable dispersion of nanomaterials and role of functionalization in maintaining its stable dispersion is indubitable (22, 23). Thus, it is a great challenge to establish the most suitable atomic organization on their surfaces so that their extraordinary properties could be retained along with quenching of the innovative and problem oriented quest.

A large class of biologically active natural products is unable to perform their pharmaceutical action in the biological atmosphere. The main cause of their retarded action is due to their poor water solubility or complete water insolubility. Carbon nanomaterials (CNMs) have provided us an opportunity to impart artificial solubility in these biologically active compounds. These CNMs has many superior properties such as low-weight, very high aspect ratio, high electrical conductivity, extraordinary mechanical, optical, and thermal properties (24, 25). Graphene, GO and CNTs have built broad interest in most areas of science and engineering because of their extraordinary physical, mechanical, thermal and optical properties (26). The use of graphene and carbon nano tubes is one of the greatest achievements in recent times which enable us to deliver biologically active molecules into the target organs without changing the atomic organization of the molecule. Graphene have tremendous potential to be used as an effective drug carrying vehicle due to its potential to penetrate plasma membrane (27) and an effective cellular uptake (26) (28), when loaded with water insoluble drugs (29-31). Idea of modifying surface of these CNMs at nano scale has been satisfactorily exploited in recent times for the efficient drug delivery. Surface modification of the graphene is favored due to the presence of hydroxyl, epoxy, and carboxylic groups; revamping the surfaces of these nanomaterials with the hydrophilic moieties make them excellent drug carriers.

Among the different members of graphene family, the most commonly used member for the drug delivery purpose is GO. The preference which goes to GO is due to its surface, which bears epoxy hydroxy and acidic groups in ample numbers in comparison to other members of the family. GO is predominantly prepared using a standard or modified Hummers’ method, employing prolonged exposure to strong oxidants such as sulphuric acid, sodium nitrate and potassium permanganate, applied to bulk graphite, where the oxidation also aids the exfoliation of the graphite (24).
These functional groups on the surface of GO could be used to functionalize hydrophilic moieties on their surface. These hydrophilic moieties are helpful in making hydrogen bond with water molecules, which helps in carrying drug molecule inside the biological systems. Nanocarriers such as graphene, GO or CNTs provide large surface areas to drugs for their loading. Extended pi-cloud in these carbon nano carriers offers a huge platform for the aromatic drugs to attach through the surface via Pi-Pi stacking. As the surface area of the nanomaterials is very high, a huge amount of drug could be loaded onto the surface (24). The enhanced solubility and drug carrying capacity enhances the therapeutic effect of the drug against the target cells. Functionalization of nanomaterials with requisite moieties on the surface is arduous as well as innovative task for a chemist. Covalent functionalization can be achieved by various techniques (48). These techniques include functionalization using cationic, anionic, and radical moieties for the surface revamping or by using click chemistry. Along with polymers there are many incidents where graphene sheets have been functionalized with bimolecular, metal monohybrids macromolecules for the better exploitations of these sheets. Functionalization of nano surfaces with non-covalent and other functionalization approaches has also been analyzed recently. Covalent functionalization is a vital method to alter the state of bond connectivity. For the better application of these nanomaterials covalent functionalization is adopted, the surface of graphene is disrupted by changing sp2 carbon atoms to sp3 carbon atoms, and hydrophilic moieties such as Poly vinyl alcohol are functionalized into the surface. A wide range of surface modification approaches have been adapted in order to make the graphene sheet more exploitable for the drug delivery application. Since aggregation of nanomaterials remarkably reduces the aspect ratio and then diminishes the classical properties of nanocomposites (24), the surface modification of nanomaterials becomes more important for the nano chemists. In principle, mode of functionalization on nano surface primarily depends on the nature of problem and intended exploitation of surface. In recent times, various efficient methods have been developed for the functionalization, most of which come under either covalent or non-covalent functionalization. Covalent and non-covalent functionalization on the nano surface is possible due to unique structural variations inherited by these materials. Covalent interactions on the surface are favoured due to the presence of hydroxyl, epoxy, and carboxylic groups are considered to be the best moieties for the functional group conversion and covalent interactions (32). Along with this, presence of sp2
hybridized π-network provides opportunity for non-covalent interaction between CNMs and host species. Drug is loaded into the surface through π-π stacking. Aromatic drug are preferred over other nonaromatic drugs. The polymer functionalized graphene sheet is able to load a huge amount of drug moieties onto its surface. The large loading capacity is attributed to the large surface area of the graphene sheets. There is a huge reservoir of biologically active molecules in nature. The challenge to exploit them for their enhanced pharmaceutical action could be accomplished by using these CNMs. Excellent aromatic rings favors their loading onto the nano surfaces, and functionalized hydrophilic wings carry these loaded drug molecules inside the biological atmosphere and deliver them to the target organs (33).

Various approaches have been adapted and developed in order to acquire such sophistication on the nano surfaces, these strategies are based on abstract philosophies of the material. In principle, mode of functionalization on nano surfaces primarily depends on the nature of problem and intended exploitation of surfaces. In recent times, various efficient methods have been developed for the functionalization, most of which come under either covalent or non-covalent functionalization. Covalent and non-covalent functionalization on the nano surface is possible due to unique structural variations inherited by these materials. Covalent interactions on the surfaces are favoured due to the presence of hydroxyl, epoxy, and carboxylic groups are considered to be the best moieties for the functional group conversion and covalent interactions (32). Along with this, presence of sp² hybridized π-network provides opportunity for non-covalent interaction between CNMs and host species.

4.2 Nanomaterial Based Drug Delivery

CNMs provide advanced drug deliverance systems equipped with superior targeting and enhanced action oriented nature which makes them an outstanding in-vitro and in-vivo drug delivery candidate for the better handling of diseases in comparison to traditional drug delivery system. Scope of interference on nano surfaces provides excellent opportunity to manipulate their characteristics physiochemical properties. Development of a competent drug delivery system and its importance have been previously explained and revised by several authors (35-36). Enthralling unique physiochemical properties, the carbon nanomaterials enable us to generate captivating ideas and opportunities to identify, analyze, and medicate
diseases more efficiently. In addition, it is obliging in the better understanding of various cellular mechanisms with the help of biosensors.

Importance of CNMs, especially of CNTs and GOs in various biological and medicinal applications is an indirect function of their numerous outstanding properties exhibited only by these appealing nano family members. Their potential to penetrate plasma membrane (37) and their effective cellular uptake (38-39) loaded with various drugs including water insoluble drugs (40-42), biosensors (43-44), bioactive molecules such as nucleic acids (45), proteins (46), genes (47-49) and lipids (50) activates quest of scientific community to take advantage of this unique nanomaterial. In addition, CNMs have been extensively used in the tissue engineering as a scaffold for the development of biological alternatives that refurbish or replace whole or a portion of tissue owing to their capability of providing microenvironment similar to that of biological extracellular matrix (51). Various members of this family have been previously exploited for binding and propagation of mammalian cells (52), including human neural and stem cells (53-54).

Relevance of CNT to the field of drug delivery has fascinated increasing research interest among scientific community in last decade (55). Binding of drug molecule on the nano surface is one of the challenging tasks during the process so that transportation and delivery could be achieved successfully. A variety of strategies have been developed to attach various drugs on CNTs via either covalent linkage or non-covalent adsorption. Pristine CNTs are hydrophobic and cannot be dispersed homogeneously in most solvents and physiological solutions. Therefore, functionalization of CNT is required for a good solubility and a benign biocompatibility. Bianco et al. reviewed the achievement of CNT in drug delivery with a specific emphasis on the different approaches to the biofunctionalization of CNT with bioactive peptides, proteins and nucleic acids (56). In comparison with CNT, GO exhibits some merits like low cost, two external surfaces, facile fabrication and modification, and absence of toxic metal particles (57). GO with all sp² carbon atoms exposed on its surface have an ultrahigh surface area for efficient drug binding (58). Moreover, the planar GO sheets can be easily complexed with functional NPs for potential multi-modal imaging and therapeutic applications.
Present crisis with anticancer drugs can be recapitulated with their deprived cellular entry and underprivileged solubility in biological environment resulting into their poor therapeutic impact. Mesmerizing findings associated CNTs and GO has added a new opening into the scenario. In recent developments, many successful efforts have been made by research community for the development of efficient delivery systems with the enhanced ability of cellular uptake and solubility in biological environment. Various biocompatible functionalized CNMs have been synthesized in this regard. Basic objective of functionalization is to render high aqueous solubility to enhance their ability to cross biological cell membrane against the existing hindrance for the drug by adding hydrophilic and biocompatible moieties on the nano surfaces. Highly hydrophilic and biocompatible moieties have been used to facilitate artificial solubility for doxorubicin and have shown optimistic results in their laboratory trials as an effective anticancer drug. High aspect ratio of CNMs offers superiority over existing delivery routes, as the high surface area provides multiple attachment sites for drug targeting ability of GOs and CNTs in the attachment and delivery of aromatic ring containing anticancer drugs such as doxorubicin (DOX) and camptothecin (CPT) can be seen as a reflection of π-π stacking between CNMs and drug π-clouds (48). The remarkable property of these drugs is to possess sp² hybridized six and five member, π-cloud containing rings which offer biocompatible interaction between drug and CNM delivery vehicles. Theoretical attempts have also been made in order to elaborate non-covalent interaction between CPT and nanosheets by using density functional theory (DFT) calculations and other computational tools and shown that noncovalent adsorption of CPT is thermodynamically favoured onto the nanosheets (59).

However, the cytotoxicity of CNTs is controversial due to contradictory reporting. One of the studies showed that PEG-functionalized GO exhibited minor in-vitro toxicity even subjecting to a high concentration of 100 µg/mL (41). Contrary to this, human fibroblast cells and mice has shown 50µg/mL of pristine GO can exhibit chronic toxicity and lung granulomas formation (42). Many researchers have shown that the designed functionalized CNTs are able to reduce cytotoxic effects (43-45), and at the same time improve biocompatibility (44-46) thus, offering the potential exploitation of nanotubes for drug administration. On the other hand, more recently graphene and its derivatives have been enormously investigated in the biological
applications because of their biocompatibility, unique conjugated structure, relatively low cost, and availability on both sides of a single sheet for drug binding (39, 47).

4.3 Functionalization of Carbon Nanomaterials

Functionalization plays a crucial role in developing high performance of CNMs for the drug delivery purposes. Surface revamping is crucial for the better dispersion and alignment of CNMs and strong interfacial interactions between CNMs and drug molecule. The functionalization of nanosheets or tubes is best approach to deliver such molecule with more efficiency. Most of the functionalization approaches developed at present could be categorized into two categories: covalent and non-covalent functionalization. These functionalization methods will be summarized here.

4.3.1 Covalent Functionalization Approach

Covalent functionalization is a crucial method to alter the state of bond connectivity. In the case of covalent functionalization, the translational symmetry of CNTs and graphene is disrupted by changing $sp^2$ carbon atoms to $sp^3$ carbon atoms, and the properties of nanofillers such as electronic and transport are influenced. In covalent functionalization, the functional units form the covalent linkage with skeleton of the graphene or CNTs. There are several covalent approaches to functionalize these carbon nanomaterials.

4.3.2 Functionalization Using Cationic, Anionic, and Radical Polymerization

Introduction of suitable functional group is a first step towards growing polymers from the surface of the CNTs or graphene followed by monomer polymerization. Acid groups, which are generated on the graphene and CNTs by oxidization procedures, may be used for the attachment of an initiator via esterification or amidation (60-61). Many polymerization methods, e.g. atom transfer radical polymerization (ATRP), reversible addition fragmentation chain transfer polymerization (RAFT), nitroxide mediated radical polymerization (NMRP), anionic polymerization, and ring-opening polymerization (ROP) techniques have been used to factionalize CNTs or graphene.
4.3.3 Covalent Functionalization Using Click Chemistry

Due to the strong interlayer cohesive energy and the surface inertia of graphene, development of powerful and reliable strategies to covalently functionalize graphene for efficient grafting and achieving precise interface control remains a challenge. For practical applications, carbon nanomaterials (CNMs) (graphene/CNT) functionalization needs to be controlled. So there is a real need for original chemical reactions which allow the modification of CNMs in a simple way. Cycloaddition reactions can play a significant role as an emerging route in this direction. Cycloaddition reactions generally proceed between two unsaturated entities to give a cyclic product in an atom-economic manner, where the flow of electrons takes place from the highest occupied molecular orbital (HOMO) of one molecule to the lowest unoccupied molecular orbital (LUMO) of the other molecule. The functionalization of CNMs by means of cycloaddition reactions plays an important role in this regard and covers a wide range of reactions, such as 1,3-dipolar cycloaddition of azomethine ylides (62), CuI-catalyzed azide-alkyne cycloaddition reactions (63), (4+2) Diels–Alder reactions (64), (2+1) cycloaddition reactions (65) and other cycloaddition reactions. The present section focuses on covalent functionalization of CNMs using copper catalyzed azide-alkyne “click” reaction a variation of Huisgen 1,3-dipolar cycloaddition reaction between terminal azides and acetylenes. Through this reaction, an enormous variety of molecules can be coupled onto CNMs in a very controlled manner, and could be utilized for many potential applications from nanoelectronics to bio-applications.

4.3.4 Functionalization with Biomolecules

The Cu (I)-catalyzed azide-alkyne cycloaddition reaction popularly known as the "click" reaction, has been extensively exploited in molecules/macromolecules build-up, and has offered tremendous potential in the design of nanomaterials for applications in a diverse range of disciplines, including biology. The efficient delivery of active pharmaceutical agents to specific organelles like mitochondria and lipid bodies, employing nanocarriers developed through the use of click chemistry, constitutes a continuing topical area of research (66). In this section, we highlight important contribution of click functionalized CNMs in the biological system including drug delivery system.
4.3.5 Functionalization with Metal Nanohybrids

Owing to their electrical (67, 68) magnetic (69, 70) and catalytic properties (71, 72), metal NPs with well defined shapes and sizes have received considerable attention. NPs with a controlled size or shape are typically synthesized by reducing metal precursors in the presence of organic surface-capping agents, however, these surface-capping agents often hinder further applications of the NPs, prohibiting dispersion in other kinds of solvents or making surface functionalization more difficult (73). Techniques that facilitate simple and universal surface modification are important to various applications (74-77), in particular, the immobilization of shape controlled NPs on a support can further extend the potential applications. Metal catalysts supported by carbon nanomaterials (CNMs) have excellent characteristic properties, owing to their high surface area, thermal stability, and porous surface, which act as a scaffold to prevent the agglomeration of the immobilized metal particles.

Over the past decades, several studies have been reported on the use of polymer based hybrids, which allow achieving the desirable properties for the resulting materials (78-82). Two main categories “grafting to” and “grafting from” approaches have been reported for the covalent grafting of polymers onto CNMs. The “grafting to” approach is based on the attachment of as-prepared or commercially available polymer molecules on the CNMs surface by chemical reactions, such as amidation, esterification, radical coupling, etc. The polymer must have suitable reactive functional groups for preparation of nanocomposites in this approach. The “grafting to” approach allows grafting a polymer with functional end/side groups (e.g., -OH, -NH₂, -COOH and -COCl) onto CNMs. So this technique is easy to carry out with various polymers including linear as well as dendritic ones, whereas the grafting efficiency is always low owing to the steric hindrance of the pre-grafted macromolecular chains. In case of “grafting from” approach, the polymer is bound to the CNMs surface by in-situ polymerization of monomers in presence of reactive CNMs or CNM supported initiators. The main advantage of this approach is that the polymer-CNMs nanocomposites can be prepared with high grafting density. This approach has been used successfully to graft several polymers onto CNMs via radical, cationic, anionic, ring-opening, and condensation polymerizations the click coupling of CNMs with macromolecules was found very effective for controlling the material
properties and achieving high performance materials. Covalent functionalization of alkyne-decorated SWNTs with well-defined, azide-terminated polystyrene polymers was accomplished by the Cu (I)-catalyzed $\left(3 + 2\right)$ Huisgen cycloaddition (83).

### 4.3.6 Non-Covalent and Other Functionalization Approaches

The drawback of covalent functionalization is that the perfect structure of CNTs or graphene is destroyed, resulting in significant changes in their physical properties. H-bonding and $\pi-\pi$ stacking play an important role as a noncovalent functionalization which has significant advantages over covalent functionalization as it enhances the solubility and assembly without effecting $\pi-\pi$ conjugation of the skeleton of the CNTs or graphene (84, 85). Water-soluble pyrene derivative is used to make stable aqueous dispersions of graphene sheets using non-covalent strong affinity of $\pi-\pi$ stacking (86). Long-range self-assembled monolayers of graphene are produced using $\pi-\pi$ interaction for various application (87,88). Very recently, several non-covalently functionalized graphenes with polymer via multiple $\pi-\pi$ stacking, H-bonding, and hydrophobic interactions have been reported (89). A simple water solution processing method is used for the preparation of poly (vinyl alcohol) (PVA) nanocomposites with GO and an increase in the mechanical property. The strong noncovalent interaction through H-bonding has been attributed for such dramatic increase of mechanical property (89).

On the other hand, non-covalently nafion-functionalized transparent conducting films of graphene have been fabricated via the reduction of GO/nafion dispersant using hydrazine; actually this nafion-functionalized graphene is produced by hydrophobic interaction of nafion with graphene surface with exfoliation of the graphene by an electrostatic mechanism. Stable dispersion of reduced graphene in various organic solvents was achieved via noncovalent functionalization with amine-terminated polystyrene polymers by sonication (85).
4.4 Experimental

4.4.1 Preparation of Graphene Oxide

GO was synthesized in laboratory by using modified Hummer’s method. In this method natural graphite powder was treated with various oxidising agents. These oxidising agents help in the exfoliation of graphite sheets along with producing functional groups on the surface by oxidation, viz. –OH, -O- and –COOH. These functional groups can be used for the functionalization of the surface with suitable hydrophilic polymer (PVA in this case).

In this method, Graphite powder (3 g) was put into 80 °C solution of concentrated H₂SO₄ (97 %, 12 mL), K₂S₂O₈ (2.5 g), and P₂O₅ (2.5 g). The mixture was kept at 80 °C for 6 h using a hotplate. Successively, the mixture was cooled to room temperature and diluted with 0.5 L of de-ionized water and left overnight. Then the mixture was filtered and washed with de-ionized water using a 0.2 µm Nylon filter to remove the residual acid.

This pre-oxidized graphite was then oxidized by the Hummers’ method. In which 1 g of the pretreated graphite and 0.5 g of NaNO₃ were placed into a flask. Then, 25 mL of H₂SO₄ was added with stirring in an ice-water bath, and 3 g of KMnO₄ was slowly added over about 1 h. Stirring was continued for 2 h in the ice-water bath. After the mixture was stirred vigorously at room temperature for 2 days, 100 mL of 5 wt% H₂SO₄ aqueous solution was added over about 1 h with stirring and
the temperature was kept at 98 °C. The temperature was reduced to 60 °C, and 3 mL of H$_2$O$_2$ (30 wt% aqueous solution) was added, and the mixture was stirred at room temperature for 2h. The oxidation product was purified by rinsing with a 10% HCl solution, repeatedly washing with plentiful de-ionized water, and filtering through a 0.2 µm Nylon membrane (90-94). The product was dried under ambient condition for overnight. The preparation can be depicted as follows (94).

Fig. 4.2 Schematic Representation of GO Synthesis

4.4.2 Preparation GO-PVA Nanocarrier

A hydrophilic carrier or polymeric chain could impart artificial solubility on GO surface, and can be utilized for the development of efficient carrier for the drug. Functionalization of PVA on GO surface becomes possible due to the presence of functional groups (94). The GO was functionalized with PVA in a carbodiimide-activated esterification reaction. In this experiment, 50 mg purified GO was dissolved in 15 mL DMSO and sonicated for 30 min to obtain a homogeneous brown-colored solution. The catalysts, DCC (2.3 g, 11 mmol) and DMAP (0.17 g, 1.4 mmol) were gradually added into the flask and stirred for 10 min. Then, a solution of PVA in DMSO (100 mg/mL, 5 mL) was added, and the resulting mixture was stirred at 50 °C for 3 days. After the reaction was terminated, the suspension was filtered over a 0.2 µm PTFE microporous membrane, and the obtained solid was washed thoroughly with DMF and acetone. In order to eliminate the unreacted PVA, the solid was dissolved in hot water, and the suspension was filtered using a 0.2 µm nylon membrane. After washing the filtered residues with a large amount of hot water, the PVA functionalized GO was obtained and dried in a vacuum oven (90-93). GO surface could be depicted as follows (94).
4.4.3 Drug Loading on the Functionalized GO

5 mL of 0.5 mg/mL GO-PVA in DI water was separately mixed with 0.5 mL of 0.1 mg/mL QSR DMSO solution and stirred at room temperature for overnight. Excess QSR precipitated as solid was removed by centrifugation. The supernatant was filtered through a 0.8 μm filter to fully remove any solid. The solution was then dialyzed (molecular weight cut-off (MWCO) = 3 kDa) against distilled water for 2 days to remove the small amount of solubilized free QSR and the sample was preserved in darkness at 4°C (93).

4.4.4 Spectral Analysis

Raman spectra of GO was recorded using 532 nm excitation from a diode pumped frequency doubled Nd:YAG solid state laser (model GDLM-5015L, Photop Swutech, China) and a custom-built Raman spectrometer equipped with a SPEX TRIAX 550 monochromator and a liquid nitrogen cooled CCD detector at Jawaharlal Nehru Centre for Advanced Scientific Research (JNCASR), Bangalore. Infrared spectra (FT-IR) of the samples were recorded with FT-IR spectrometer (Perkin Elmer Spectrum Version 10.03.06) in the range of 4000~450 cm⁻¹ using KBr disk method at Centre of Bio-Medical Research (CBMR), Lucknow. UV and AFM images (Make Naio) of the samples were recorded at University of Petroleum and Energy Studies, Dehradun, India.
4.5 Results and Discussion

4.5.1 Characterization of GO and GO-PVA

GO sheets were obtained by the exfoliation of graphite and further characterized by different spectroscopic techniques such as Raman spectrometry, AFM, and FT-IR spectrometry. Results have clearly shown the synthesis of GO by the exfoliation of graphite. In the Raman spectra of GO sample, peculiarly two bands in the range of 1610.63 cm\(^{-1}\) and 1364 cm\(^{-1}\) can be seen; these bands are respectively known as G and D bands and are the characteristic peaks for the GO (Fig. 4.4). Presence of functional groups on the surface of GO is confirmed by the FT-IR spectroscopy. In the FT-IR spectra absorbance peaks at 1057 cm\(^{-1}\), 1379 cm\(^{-1}\), 1614 cm\(^{-1}\), 1726 cm\(^{-1}\) can be attributed to C-O stretching (epoxy or alkoxy), C=C stretching, C=O stretching (carboxyl) respectively. Broad peak in the range of 3200-3500 cm\(^{-1}\) can be assigned to the hydrogen bonded –OH and –COOH groups on the surface (Fig. 4.4). AFM images confirm that samples are comprised of isolated few-layer sheets of GO. Samples have dimensions of several nanometers (minimum obtained, 281nm and 301 nm) and a thickness of few micrometers and are very suitable for the drug loading and drug delivery (Fig. 4.5).

Functionalization of GO was confirmed by Raman, AFM and FT-IR spectrometry. On comparing the Raman spectra of the GO and GO-PVA certain characteristic changes can be seen clearly. The functionalization on the GO surface can be identified by the relative shifts in the D and G bands. It is evident that D band gets downshifted after the functionalization on the surface while at the same time it has been observed that the G band gets positively shifted after functionalization (Fig. 4.6). It is clear from the spectra of GO-PVA that after functionalization characteristic D band in GO-PVA downshfits by 4.52 cm\(^{-1}\) on the other hand, the G band gets upshifted by 1.18cm\(^{-1}\), confirming functionalization of GO sheets, and are in complete agreement with the previous reports (96).

In addition to this, FT-IR spectra of GO-PVA also confirm functionalization of the GO surface (Fig. 4.7). Three characteristic changes can be seen after functionalization. On comparing FT-IR spectra of GO with GO-PVA, the first change appears in the peak of hydroxy groups, in the functionalised GO the surface COOH
groups gets consumed by the PVA in order to form the ester linkages. The decrease in the number of COOH groups leaves non hydrogen bonded –OH groups behind on the surface, consequently a sharp peak for the OH groups appears in this region, this peak may also be attributed due to the –OH groups of the PVA. Second change can be seen in the region of 2800 to 3000 cm\(^{-1}\), where prominent peaks for the methylene (–CH\(_2\)–) stretching vibration can be seen clearly at 2914 cm\(^{-1}\) and 2850 cm\(^{-1}\). The third characteristic change can be seen by the appearance of peaks for the ester linkage, which appears due to the reaction of the PVA with surface –COOH groups. This reaction leads to the formation of ester linkage on the surface of the GO, and can clearly be seen in the spectra of the GO-PVA.
Fig. 4.4 Raman and FT-IR Spectra of GO

Fig. 4.5 AFM Images of GO
Fig 4.6 Raman Spectra and AFM Image of GO-PVA

Fig 4.7 FT-IR Spectra of GO-PVA
4.5.2 Drug Loading

Loading of drug can be confirmed by comparing the UV spectra of GO-PVA and GO-PVA-QSR. Synthesized GO-PVA-QSR was evaluated for the UV-Visible analysis. Scans were performed over a wavelength range of 200 to 520 nm under laboratory conditions. 1 mg of GO-PVA-QSR was thoroughly dissolved in the solvent DMSO, owing to its better solubility in it. Two characteristic peaks were recorded for quercetin, and can be seen from 330 nm and 385 nm respectively. The results obtained for the UV spectra confirmed the loading of isolated QSR molecules in the developed nano carrier. Results for the loading of QSR are in complete agreement with previous reports on the UV spectra of the QSR (90). While the spectra of GO-PVA shows mere a hump ~ 250nm similar to previous report on such samples (92). In the spectra of GO-PVA-QSR characteristic peak for the QSR molecules can be seen clearly while in GO-PVA this peak is completely absent confirming loading of the isolate form the plant successfully (90-94).

Fig 4.8 UV Absorption Spectra of GO, GO-PVA-QSR and AFM Images of GO-PVA-QSR

AFM images confirm that samples comprise of isolated few-layer sheets of PVA-QSR-GO. Samples have lateral dimensions of several nanometers (minimum obtained (281nm and 301 nm) and a thickness of few micrometers. The results explore that the developed nanocarriers are suitable for the successful delivery of the
drug molecules in the cancer cell lines as the average size of cancer cell is considered to be 10000nm to 20000nm (95).

4.6 Conclusion

The GO was successfully prepared in laboratory and the synthesis of GO was confirmed with the help of various spectral analysis. The evidence for the synthesis of GO was confirmed by the FT-IR, Raman, and AFM spectra of the prepared sample. Raman spectra have provided solid results for the synthesis of GO, revealing peaks for corresponding G band and D bands. Similarly the functionalization of the GO sheets was confirmed by IR and Raman spectroscopic techniques. Isolated natural product was successfully loaded on the GO sheets and confirmed by the UV spectra of the corresponding samples. AFM images have shown that the size of drug loaded GO is suitable for the penetration of the cancer cells and can be used for the effective drug delivery.
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