Research Article

Preparation and Evaluation of PEG Functionalized MWCNTs- Irinotecan Conjugate

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ABSTRACT

Anti-cancer drugs are very powerful, the drug performance is decreased via their systemic toxicity, maximum tolerated dose, drug loading, slender therapeutic window, formulation cost and restricted cells entry. CNTs were proposed and explored as progressive nano scaffolds for diagnostic and in most cancer drug shipping areas. Our research studies offer a new proposal of developing a secure and controlled drug delivery conjugate gadget by using MWCNTs. On this examine, functionalized -MWCNTs composite was successfully prepared by way of combining MWCNTs with PEG polymer and applied as a drug carrier for Irinotecan. The produced PEG functionalized – MWCNTs- Irinotecan conjugate subjected to specific characterization techniques together with Scanning Electron Microscopy (SEM), Fourier- transform infrared spectroscopy (FT-IR), Thermo-gravimetric Analysis (TGA), Particle Size, Zeta potential, Drug entrapment efficiency, In-vitro drug release, and Drug release kinetic studies. Our study demonstrated that, PEG functionalized MWCNTs -Irinotecan conjugate had been promising candidate for drug shipping. The conjugate suggests maximum drug entrapment efficiency, and the sharp drug release was discovered at 1 hr. and showing sustained launch of drug up to 12 hr.

Keywords: Anticancer, Pristine MWCNTs, PEGylation, Conjugate, Noncovalent Functionalization.

INTRODUCTION

Cancer interpret as the unlimited increase of cells that invade, resulted in destroying the normal tissues. Unlimited cell growth and division in several mutagenesis steps that leads the cancer cells to acquire self- sufficiency in development of signals and resist the signals from the normal cells that could, in any other case halt their proliferation or result in apoptosis. Tumours will also evolve by additional extra help by using interactions amongst surrounding stromal cells, including which progress the angiogenesis, and break away from the immune system, resulting in metastasis. It turned into predicted that each year, 14 million new cases of carcinoma are diagnosed and 8.9 million mortality rate of the chronic (ongoing) illness. Breast, cervical, gastrointestinal tract, Lung, and liver cancers are taken into consideration the most frequent etiology for the cancer-related demise. Although most cancers research technology have advanced surprisingly, techniques to detect on most cancers at an early level and cancer-unique remedy that does not damage normal cells[1]. In this modern world, cancer is the one of the most challenging disease of cutting-edge instances because its therapy entails distinguishing ordinary healthful cells from affected cells[2]. Irinotecan is an antitumor enzyme inhibitor basically used for the treatment of colorectal cancer. Irinotecan (7-ethyl-10-[4-(1-piperidino)-1-piperidino) carbonyloxy-camptothecin) is an inhibitor of topoisomerase I. It is a derivative of camptothecin that impede the motion of topoisomerase I. While activated by using tissue carboxylesterses, it really works by stopping the DNA from unwinding. Topoisomerase I, it's far an enzyme chargeable for maintaining DNA in its right form while cells are dividing. Irinotecan averts the relegation of DNA strand by means of binding to topoisomerase I – DNA complex, and double- strand DNA breakage results in cell death [3].

The "nano" is a Greek word, that means elf (small); clinical treatment at the nano stage (atomic level) with the assist of unique clinical gadgets is referred to as nanotechnology, ultimately which has become a familiar in last three to four years. The phrase "nanotechnology" coined in 1974 with the aid of Norio Taniguchi, in japan. Scientist assert that," Nanotechnology" particularly includes the development steps of the separation, consolidation and deformation of materials through one atom or one molecule. Nanotechnology is a significant subject which scrutinize many records approximately the design and properties of substances.

Carbon is the 15th maximum ample element within the crust of the earth. Having a tetra valency and therefore a hybridization of sp3, carbon can shape a huge form of crystalline configurations. Graphite, diamond, Buckminster fullerenes, carbon nanotubes, graphene and around 500 hypothetical allotropes comes under the allotropic types of carbon [4].

Carbon nanotubes (CNTs) are characterised as a tube which are rolled up graphene sheet. Carbon nanotubes (CNTs) are apparently the magnificent member of the fullerenes family. Fullerenes contain any molecule made absolutely of carbon atoms, fashioned as a sphere, an ellipsoid, or a tube[5]. Nanotubes have the best chemical composition and atomic bonding configuration but

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exhibit possibly the most excessive range and richness among nanomaterials in structures and structure-assets members of the family [6]. Initially, to build carbon nanotubes the arc discharge method was employed. This method was recognised and deployed for the synthesis of carbon filaments and fibers. afterward other strategies including laser ablation or chemical vapor deposition (CVD) had been tested for the production of carbon nanotubes. In reality, these are the three major manufacturing methods [7].

Carbon nanotubes (CNTs) appear just as they bang: minute tubes made of carbon, indubitably, it is similar carbon that is present in human beings as well as in bucky balls or graphite! But, their exciting physico-chemical aspects, especially high surface location, easy of drug loading, ultra-light weight, tunable surface chemistry, pseudo fragrant structure, fluorescence detectability and optoacoustic effects lead them to a capacity asset for the depot of gnostic nanocarriers[8].

Carbon nanotubes (CNTs) are one of the allotropes of carbon. A CNT is a one – atom thick sheet of graphite (known as graphene) rolled up into a unbroken cylinder with diameter of the order of a nanometer. In 1952 the Carbon nanotubes (CNTs) had been first found and defined by Radushkevich and Lukyanovich. And later in 1976 the single and double walled carbon nanotubes were observed. The first scientist Iijima invented and characterise the multi walled carbon nanotubes (MWCNTs). The length-to-diameter ratio of nanostructure (MWCNTs) goes above 10,000. Such carbon tubular molecules have invigorated properties that sword them possibly useful in wide variety of application programme in structural, thermal, mechanical, electronic, optical, biomedical and other champaign of science, engineering & medication [9].

Different varieties of carbon nanotubes (Based on tubes range present inside the CNTs, it is divided into 3 categories)

1. Single – walled CNTs

   Single –walled CNTs are made from a single graphene sheet rolled upon itself with a diameter of 1-2 nm. The length of single walled carbon nanotubes depends on preparation practice strategies.

2. Double –walled CNTs

   Double –walled CNTs are made of two concentric graphene sheets, which the outer tube encloses the internal tube.

3. Multi – walled CNTs

   Multi – walled CNTs are made of multiple layer of graphene sheets rolled upon with a diameters of 2-50 nm depending at the variety of graphene tubes with approximate inter-layer distance of 0.34 nm[10, 11, 12].

MWCNTs own a π-conjugative shape with an extraordinarily hydrophobic externality, due to high molecular weight they have rarely soluble in natural solvents and their tendency to entangle and form 3-D networks thru persistent Van der waals interactions. The most of the accepted artificial methods which produces CNTs, which yields a mixture of diverse diameters and chiralities of nanotubes that are normally contaminated with metallic and amorphous impurities. further, the commercialized CNTs are supplied in the form of closely entangled bundles, ensuring the inherent difficulties in dispersion.

In order to solve those problems, the surface properties of CNTs are modified by using functionalization techniques. These functionalization approaches can be absolutely divided into chemical (covalent functionalization) and physical (Non- covalent functionalization)[13].

The CNTs special properties such as high drug loading, cellular uptake, and thermal ablation, render them useful for cancer treatment. The pristine CNTs cytotoxicity is due to CNTs residual metal catalyst (impurity) and insolubility property. Consequently, to introduce the CNTs as a drug carrier into biological structures, for CNTs applications, to make CNTs soluble and improve the biocompatibility, the CNTs need to be functionalized. Similarly, through functionalization process, the drugs can be attached to CNTs, where functionalized CNTs serve as a transporter of sanative agents. The fundamental mechanism of non-covalent functionalization through the physical interaction. Special molecules will attach to the basic structure of CNTs via physical interactions. Adsorption, absorption, attachment and filling of different molecules on CNTs mediated by exceptional forces including π-stacking interactions, hydrogen bonds, van der Waals force, and electrostatic force. These are the primary foundation of functionalization techniques[14].

The coating of amphiphilic surfactant molecules or polymers (polyethylene glycol) to CNTs will be carried out in non-covalent functionalization technique. CNTs having large aromatic (π- electrons) hydrophobic facet of carbon nanotubes, when interact with appropriate molecules and macro biomolecules will become best partners for non-covalent functionalization. These interactions can take location each at the inside and outside of CNTs. Once functionalization succeeded, CNTs begin to be hydrophilic and are ready to be connected with drugs or with other biomolecules (DNA, genes, proteins, enzymes, biosensors, etc.) and to deliver the drug to target cells or organs.

PEG is a amphiphilic synthetic polyether which soluble in water as well as in many organic solvents. CNTs functionalized by using amphiphilic molecules has been recognized as one of the best utility approaches to improve the dispersion of CNTs in aqueous media. PEG has been one of the most favored synthetic polyether

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molecular species for diverse purposes in bio-associated programs owning to its non-toxicity as well as its good solubility under various physiological conditions [15]. CNTs pharmacokinetics and biocompatibility can be improved by PEGylation process. PEGylation is the most efficient method which gives the escort effects on the ADMET information on the biomedical applications of CNTs[16].

As the anticancer drug used alone the efficiency is restrained by systemic toxicity, narrow therapeutic window, drug resistance and limited cellular entry. Functionalized CNTs can be used as drug conveyor to treat tumor, because CNTs can passes throughout cytoplasmic and nuclear membrane without difficulty. Anticancer drug transported by using CNTs might be liberated at the target site with intact concentration and consequently, its shows therapeutic action. The activity and concentration will be higher in tumor cell compare to drug administrated by conventional therapy. As a result, the development of systematic drug delivery system by using potent drug with increase in celluar uptake and targeting of drug. CNTs having high surface area that provides multiple sites for the attachments of drugs, so it offers a great advantages over the existing delivery systems[17-20].

In cancer therapy, to target the drug to tumor, proper amount of drug should loaded in drug delivery system with minimum adverse effects of drugs on normal and healthy tissues. Enhanced permeability and retention (EPR) effects is one of the approach to deliver drugs to tumor site. The EPR effect is due to leaky vascular structures and impaired drainage systems of tumor. The pore size of blood vessels in tumor tissues will be 100 - 800 nm. CNTs conjugated with therapeutics agents will be having pore size of 100 - 700nm penetrate through the tumor blood vessels and acts as a drug depot. Thus, the present study is focused on the preparation and characterization of Noncovalent (PEG) functionalized MWCNTs - Irinotecan conjugate. Our current objective is dual. First, we want to bind PEG on MWCNTs and MWCNTs-PEG towards Irinotecan to optimize the ideal applied concentration of drug, which can be carried out by carbon nanotubes. The second aim was to evaluate the binding capacity and in-vitro drug release from PEG functionalized MWCNTs- Irinotecan conjugate. The produced PEG functionalized MWCNTs-Irinotecan conjugate was subjected to different characterization techniques such as scanning Electron Microscopy (SEM), Fourier- transform infrared spectroscopy (FT-IR), Thermo-gravimetric Analysis (TGA), Particle Size, Zeta potential, Drug entrapment efficiency, In-vitro drug release, and Drug release kinetic studies.

MATERIALS AND METHODS

Multiwalled carbon nanotube (outer diameter 10-30nm, number of walls 5-15, length (1- 10µm) were purchased from Nano wings Private limited, Telangana. Irinotecan is a gift sample from ShangHai Henghua Trading Co., Ltd. All chemicals and reagents used were laboratory grade and they were kind gift from Samarth life science private limited, Tumkuru.

Functionalization of MWCNTs with PEG 400

500mg of pristine MWCNTs were dispersed in 100 ml PEG solution (1gm in 10 ml) with the aid of a fast-clean ultrasonic bath Sonicator for 15 mins. Removal of unbound PEG and agglomerated MWCNTs was done by centrifugation process at 7000 RPM/5 mins. Therefore, the supernatant was retained. Removal of agglomerates, and unbound PEG was performed by centrifugation and filtration done with 0.2 micro filters (Millipore) through vacuum filter and dried.

Formulations prepared by using PEG functionalized MWCNTs with Irinotecan (Loading of Irinotecan into PEG functionalized MWCNTs)

PEG functionalized MWCNTs was dispersed in Irinotecan solution (Irinotecan in water {25 mg / ml}) at varied concentrations as given in table no .01. The mixture is sonicated for 30 mins, subsequently the dispersion rotated for 24 hour by using rotor to facilitate loading of Irinotecan. Thereafter, the mixture was subjected to centrifugation at 7000 rpm for 15 minutes and then washed with methanol and followed by washing three times with deionized water and centrifuged to remove free/unbound drug. Whereas the solid was dried at 30 ◦C in a vacuum oven for 24 hours to obtain PEG functionalized- MWCNTs- Irinotecan conjugate. The resulting PEG functionalized – MWCNTs-Irinotecan conjugate was stored in vacuum desiccators for further use of studies.

Table 1: Formulation of PEG functionalized MWCNTs loaded with Irinotecan

Formulations	PEG – MWCNTs (mg)	5 Fluorouracil (mg)
F1	20	20
F2	40	40
F3	60	60
F4	80	80
F5	100	100

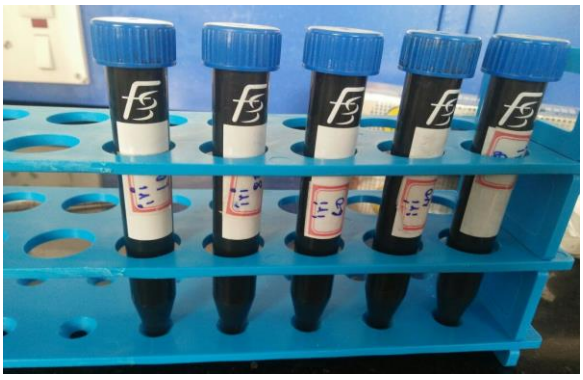

Figure 1: Dispersion of PEG F – MWCNTs in to Irinotecan solution

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Evaluation of PEG functionalized MWCNTs- Irinotecan conjugate

i) SEM studies

The scanning electron microscopic techniques are based on the irradiation of the sample by an electron source. Take sample, to break the agglomerates sonicate the sample, a small droplet of sample placed on a SEM grid by using a micro-pipette and let the solvent evaporate as quickly as possible, sample should be in dry condition for SEM analysis. Samples are allowed to be observed in pressure of -1×E-3 Pa gaseous environments in vaccum chamber, an electron beam is scanned across the specimen and the back scattered electron are detected to generate an image of the morphology or topography of the sample. The resolution of this method is commonly limited to objects larger than 1 nm.

ii) FT-IR studies

The infrared spectrum of absorption or emission of a solid, liquid or gas was obtained by using Fourier-transform spectroscopy. The sample holder should be cleaned by acetone, after cleaning make sure that splash of the acetone should not be on the instrument, so wipe by using kimwipes, take 5mg of potassium bromide make pellet, keep in pellet holder, scan range limit is 400-4000cm^{-1}, the total pressure applied to the sample should be monitored, and check IR spectra.

iii) Particle size

Particle size determination by dynamic light scattering (DLS) method. Dynamic light scattering has become the technique of choice for characterizing nanomaterials due to its speed and ability to readily characterize a statistically significant number of particles. The dispersion of sample was vigorously shaken to break up loose aggregate. As such sample (1ml) is measured with 4sided transparent disposable sizing cuvette, was measured by right angle scattering, repeat the test for three times with each two minutes time interval. The effects of dust (large particle impurities) was suppresses by noise cut features of the software data was analysed by the technique of cumulants.

iv) Zeta potential

The zeta potential is a key indicator for the stability of colloidal dispersion. The magnitude of the zeta potential indicates the degree of electrostatic repulsion between adjacent similarly charged particles in dispersion. The surfaces of the CNTs were modified by functionalization, where negative and positive charge lead to electrostatic repulsion between the molecules and stabilize the nanotube colloids. The CNTs dispersion was sonicated at 90 Hz for 15 mins in sonicator, then the solution was settled at room temperature for 24 hrs, 1ml as such sample measured in disposable folded capillary zeta potential cuvette.

v) TGA procedure

TGA technique widely used, to determine a materials characterization, as it is simple and the information of can be presented by a simple thermogram.

In STAR e software (Mettler Toledo) assign the sample name, heating rate, temperature, purge gas etc. Take empty weight of crucible (Aluminium oxide crucible, 70microlitre) and then load 1-20mg of sample. The loaded crucibles are placed on a tray and an auto sampler places them onto the microbalance in the furnace. When everything is ready then we start the experiment after completing the experiment, we have to analyse the curve and save it in folder.

vi) Drug entrapment efficiency: 21

To evaluate the average drug entrapment on CNTs, drug entrapment efficiency has to be done in three trials (n=3) to calculate the drug loading. 5 mg of PEG functionalized -MWCNTs- Irinotecan conjugate was mixed with 100 ml of phosphate buffer pH 7.4, heated at 37 ◦C and then centrifuged at 10,000 rpm for 1 hr to remove entrapped drug from MWCNTs in the formulation, 1ml supernatant solution is suitably diluted to determine the drugs concentration using by UV spectrophotometer at 254 nm, carried out at Samarth life science Pvt ltd, Vasanth narasapura, Tumkur.

$$\text{DEE\%} = \frac{(\text{Weight of loaded drug})}{(\text{weight of feeding drug})} \times 100$$

vii) *In-vitro* release studies

In-vitro drug release test conducted in three trials(n=3). All formulations release patterns was studied by using a dialysis membrane -70 (HIMEDIA). Freshly prepared Phosphate Buffer pH 7.4 used as dissolution medium. Exactly weighed amount of formulations equivalent to 5 mg of drug weighed and soaked overnight in the dissolution medium. Previously soaked drug solution filled into dialysis tube (approximately 1.2 inch in length) and the ends tied to form a pouch. The tied dialysis tube pouches were then placed 100ml of phosphate buffer pH 7.4 in conical flasks, placed in the shaking water bath and maintained at 37 ◦C with a frequency of 50 shakings per minute. 1 ml aliquots from conical flask withdrawn at regular intervals and replaced by an equal volume of the dissolution medium. The aliquots were then suitably diluted and analysed by UV-Vis spectrophotometer at 254 nm.

viii) Drug Release Kinetics Studies

To determine the drug release kinetics of all formulations, the drug release data obtained were fitted into various mathematical models

- Cumulative percent drug released v/s Time (Zero order rate kinetics).
- Log percent drug remaining to be released v/s Time (First order rate kinetics).

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- Cumulative percent drug released v/s Root Time (Higuchi matrix).
- (Amount remaining to be released)$^{1/3}$ v/s Time (Hixson-crowell erosion equation).
- Log of cumulative percent drug released v/s Log Time (Korsmeyer-pappas model)

Drug release data of all formulations were fitted in Korsmeyer-Peppas model to find out the mechanism of drug release, this model was used to study the drug release mechanism by analysing 'n' as the diffusion exponent. In the model If 'n' is below 0.45 then drug release mechanism follows Fickian mechanism 'n' is between 0.45 to 0.89 drug release mechanism follows Non- Fickian mechanism 'n' is greater than 0.89 drug release mechanism follows case- II transport or super case II transport mechanism respectively.

RESULTS AND DISCUSSION

Noncovalent functionalization of MWCNTs was carried out by coating MWCNTs with

polymer polyethylene glycol (PEG), in functionalization process shortening of length occurs due to sonication process followed by centrifugation, filtration and drying method. The MWCNTs physical properties are essentially protected by the non-covalent approach: the non-covalent functionalization of CNTs is much simpler method preserving nanotubes' SP^2 aromatic structures and their electronic characteristics.

PEG functionalized - MWCNTs Irinotecan conjugate was prepared by binding the Irinotecan with PEGylated MWCNTs by sonication, rotated for 24 hour, subjected to centrifugation, dried in vaccum oven at 30 ˚C. The resulting PEG functionalized -MWCNTs- Irinotecan conjugate was evaluated by SEM studies, FT-IR studies, Particle size, Zeta potential, Thermogravimetric analysis, Drug entrapment efficiency, In-vitro release studies, Drug release kinetic studies.

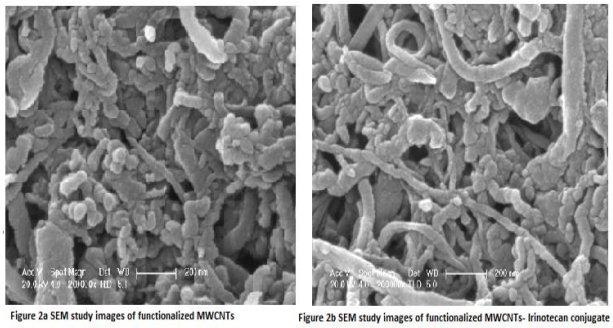

Figure 2a SEM study images of functionalized MWCNTs

Figure 2b SEM study images of functionalized MWCNTs- Irinotecan conjugate

Figure 2: SEM study images

SEM studies done at Indian institute of science, Bangaluru. PEG functionalized MWCNTs was presented in figure 2a. SEM images of carbon nanotubes shows that MWCNTs functionalized with PEG, a layer of uniform polymer was clear on the sidewall of the nanotubes. PEG functionalized MWCNTs compared with PEG functionalized - MWCNTs - Irinotecan conjugate, the PEG functionalized - MWCNTs- Irinotecan conjugate structures are quite different from PEG Functionalized - MWCNTs, in which the tube surface and diameters of nanotubes are increased as shown in figure 2b.

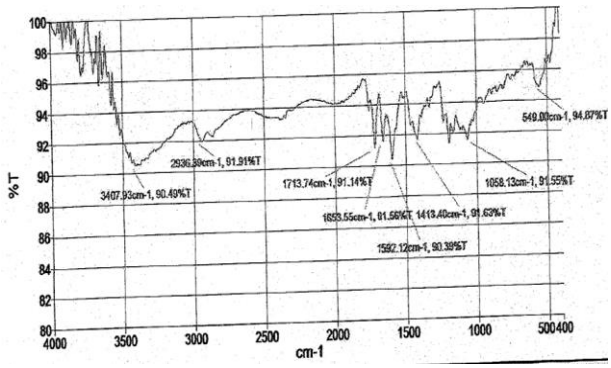

Figure 3: FT-IR graph of PEG functionalized -MWCNTs - Irinotecan conjugate

The optimized MWCNTs conjugate was characterised by the use of FT-IR spectroscopy (Perkin Elmer Spectrum II) at Bangalore testing laboratories pvt.ltd. At 4000 to 400 cm^{-1} the samples were recorded. PEG functionalized - MWCNTs- Irinotecan conjugate FT-IR was given in the Figure 3. The peak approximately at 3407.93 cm^{-1} shows characteristic of an H bonded O-H stretch, became more pronounced in PEG functionalized MWCNTs. At 1730 cm^{-1} a sharp peak was found corresponds to C=O stretching vibration of carboxylic acid. The C-O stretch vibration of ether group of PEG corresponds peak at 1100 cm^{-1}. The strong peaks 2850 cm^{-1} and 2936 cm^{-1} was seen due to C-H stretching in the PEG chain. The characteristic peaks for Irinotecan showed at 1713.74 cm^{-1}, 1413 cm^{-1} ,1231 cm^{-1}, 744 cm^{-1} due to the presence of C=O, C-H, C-O, C-Cl functional groups. The results provide more evidence of successful linkage of Irinotecan to functionalized MWCNTs.

The particle size and zeta potential of PEG functionalized -MWCNTs- Irinotecan conjugate was characterized by a laser particle size analyzer at Aimil limited, Bengaluru. The particle size of PEG functionalized -MWCNTs- Irinotecan conjugate was done in three trials. The average particle size diameter of PEG functionalized -MWCNTs- Irinotecan conjugate was found to be 222.6 nm. The zeta potential of PEG functionalized - MWCNTs - Irinotecan conjugate was done in three trials. The average zeta potential of PEG functionalized -MWCNTs- Irinotecan conjugate was found to be -9.17 mV respectively. The PEG functionalized – MWCNTs- Irinotecan conjugate TGA graph was shown in figure 4. The solid lines correspond to Thermogravimetric (TG) curve. By observing the figure 4, the initial burning temperature was found at 250 ˚C, due to amorphous carbon present in MWCNTs mixture. Around 280 ˚C CNTs starts burning themselves. At 900˚C, it was noted that sample remained behind after performing TGA. This residue remains mainly due to the presence of drug found in between and within

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the inner walls of the MWCNTs. The weight gain due to oxidation occurs in sample, since no oxidation of metal particles is expected.

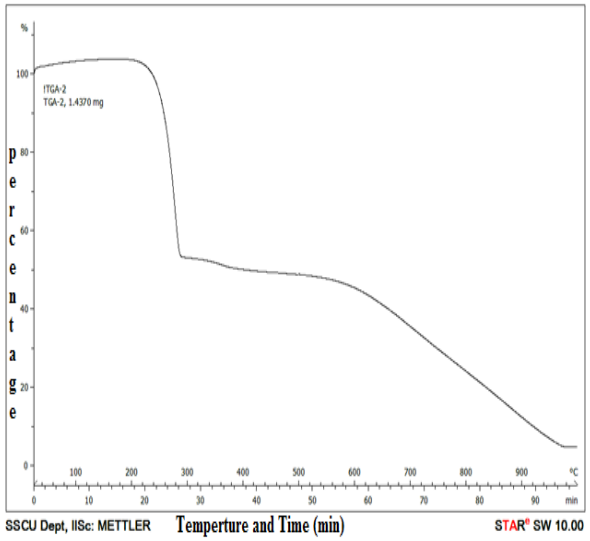

Figure 4: TGA analysis of PEG functionalized - MWCNTs - Irinotecan conjugate

All five formulations F1, F2, F3, F4, F5, showed reasonably good average percentage of entrapment with 92.08%, 72.81%, 89.65%, 85.83%, 91.36% and with the standard deviation of 2.30, 4.23, 2.76, 3.66, 2.14 respectively shown in table 02.

The *in-vitro* drug release profile shows the controlled drug release and better percentage of drug release in 12 hrs in figure 06. The release study proved that PEG functionalized -MWCNTs- Irinotecan conjugate formulations have better performance. All F1, F2, F3, F4, F5 Formulations shows better drug release performance up to 12 hrs for about 89.53 %, 64.17%, 78.13%, 71.88%, 79.48% respectively. The comparative *in-vitro* drug release profile of all the five batches showed the release of drug in controlled manner. F1 formulation showed gradual drug release pattern over a period of time with topmost drug release of 89.53%.

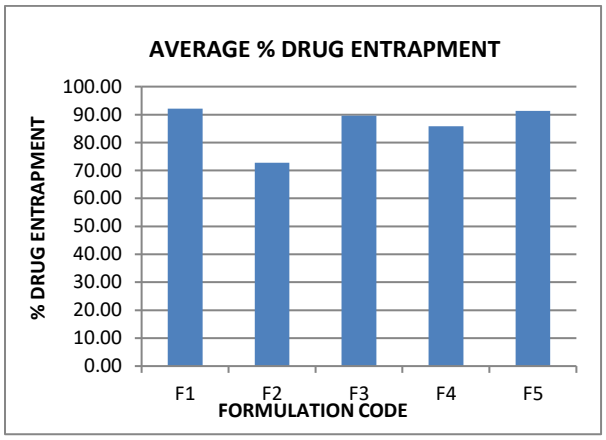

Figure 5: Bar chart for average drug entrapment efficiency

Drug release data were fitted in to mathematical model and plots of Zero order, first order, Higuchi Matrix, Hixson Crowell were plotted. The regression coefficient (r^2) vales of Zero order, first order, Higuchi Matrix, Hixson Crowell and the 'n' values for korsmayer- Pappas are tabulated in table 3, the best fit model was the first order release kinetics. The 'n' exponent value for korsmayer- pappas model for F1, F4 formulations is in between 0.45 to 0.89 indicating that these formulations drug release mechanism follows Non- Fickian mechanism and F2, F3, F5 formulations less than 0.45 indicating that these formulations drug release mechanism follows Fickian mechanism.

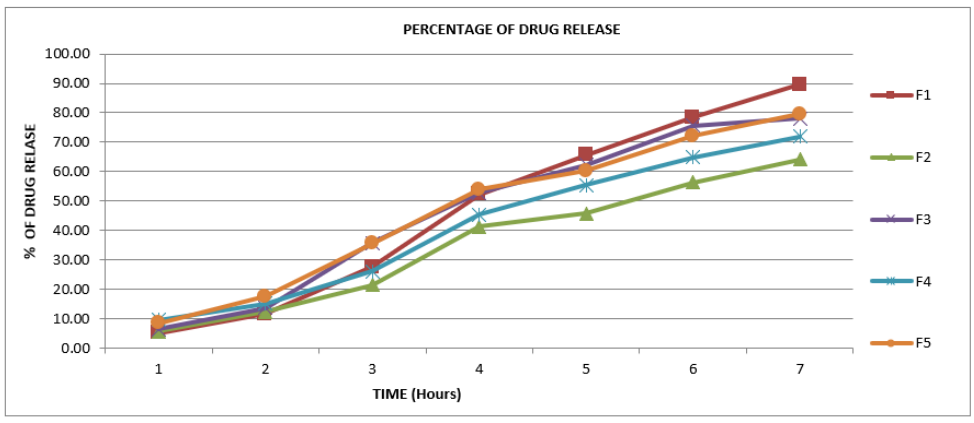

Figure 6: Average % *in-vitro* drug release graph

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Table 2: Average drug entrapment efficiency

DRUG ENTRAPMENT EFFICIENCY										
TRIAL 1			TRIAL 2			TRIAL3			AVERAGE	STANDARD DEVIATION
Formulations	UV absorbance	% of drug entrapment to MWCNTs	Formulations	UV absorbance	% of drug entrapment to MWCNTs	Formulations	UV absorbance	% of drug entrapment to MWCNTs		
F1	0.859	89.48	F1	0.892	92.92	F1	0.901	93.85	92.08	2.30
F2	0.702	73.13	F2	0.657	68.44	F2	0.738	76.88	72.81	4.23
F3	0.842	87.71	F3	0.891	92.81	F3	0.849	88.44	89.65	2.76
F4	0.828	86.25	F4	0.787	81.98	F4	0.857	89.27	85.83	3.66
F5	0.897	93.44	F5	0.856	89.17	F5	0.878	91.46	91.36	2.14

Table 3: Regression Coefficients for various models for the prepared formulations

Formulations	First	Zero	Higuchi	Hixson Crowell Mechanism	Korsmayer		Best fit model	Release mechanism
					r^2	N		
F1	0.959	0.987	0.983	0.988	0.993	0.52	**Korsmayer**	Non Fickian
F2	0.962	0.937	0.970	0.955	0.960	0.40	**Higuchi**	Fickian
F3	0.987	0.995	0.980	0.992	0.975	0.36	**Zero order release**	Fickian
F4	0.985	0.968	0.989	0.980	0.979	0.52	**Higuchi**	Non Fickian
F5	0.987	0.979	0.988	0.985	0.989	0.42	**Korsmayer**	Fickian

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CONCLUSION

MWCNTs were successfully functionalized and loaded with Irinotecan to prepare PEG functionalized MWCNTs Irinotecan conjugate. Noncovalent functionalization with PEG enhances the solubility and to reduce the toxicity of MWCNTs. Our work established easy method to prepare the formulations and PEGylation is structured method, enhances the *in-*

Vivo behavior of MWCNTs. MWCNTs plays an important role in nanomaterial research. SEM investigations proved that the MWCNTs were successfully coated with PEG and drug. The chemical groups attached to the non - covalent functionalized MWCNTs were clearly shown in FT-IR.

PEG functionalized MWCNTs Irinotecan conjugate were found to possess an average size of 226.6nm, average zeta potential of -9.17mv and to significantly enhanced the solubility and stability of Irinotecan. The results show the better drug entrapment efficiency. *In-vitro* release studies shows that PEG functionalized MWCNTs - Irinotecan conjugate possess controlled release of Irinotecan for cancer therapy. More work is under way for targeting the drug to cancer site.

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