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Effect of Statistical Analysis on drug release from Functionalized MWCNTs anticancer drug complex

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Abstract: Objectives: 5-Flourouracil loaded to MWCNTs as carbon nanotubes possess pronounced imminent in cancer therapy. The rationale is to conjugate the drug with MWCNTs and to prepare novel carrier delivery system for cancer to enhance the release of drug from F-MWCNTs-5Fu composite and to recognize conceivable effects and release of anticancer drug.

Methods: Multi walled carbon nanotubes empower covalent and Non covalent functionalization. Multi-functionalized MWCNTs loaded with anticancer drug. Formulations are considered according to DOE. The characterization studies of F-MWCNTs-5Fu composite were completed by FT-IR, SEM, TGA, and TEM. The analysis entrust the increase in particle size, and zeta potential. Drug loading efficiency and In-vitro release of drug was statistical analyzed.

Results: Our study consists of 6 factors with 3 levels, for designing the experiments L18 orthogonal array needs to be selected based on number of degree of freedom (DOF). Total number of DOF = (No of levels – 1)× No of main factors =(6-1)*1+(3-1) =11 neglecting the interaction factors, So total number of (DOF) + 1 is the minimum number of experiments. SEM and TEM images explicate that, the drug loaded to pristine MWCNTs and established by particle size analyzer. The drug entrapment efficiency outcome indicates more than 60% of drug entrapment was achieved.

Conclusion: Functionalization process help to boost the release of drug in controlled manner and upsurges the dispersion of the formulations. Prepared Functionalized MWCNTs-5Fu approaches with heightened development in medical use. FT-IR spectrum exemplifies the drug attached to MWCNTs, Functionalized MWCNTs-5Fu formulations releases the drug with good percentage and the obtained results were analyzed statistically.

Keywords: Multi walled carbon nanotubes, PEGlyation, Taguchi, Orthogonal Array, ANOVA, S/N Ratio. Multi-Functionalization.

1. INTRODUCTION:

Nanotechnology can be pronounced as the science and technology involved in the planning, production and relevance of medicines and devices on nanometre range. Nanotechnology consists of naoparticles, dendrimers, liposomes, quantum dots, fullerenes etc, are ultimately accomplish in medical field, by means of nanostructures used for analytical and therapeutic purpose.[1] Carbon based substances such as nanotubes, diamonds, nanowires, graphite are

having distinctive property and diversity of applications in electronic, tissue engineering, medical implants and devices, detectors, delivery of drugs.[2, 3]

In 1985, astounding and unintended test were done in assemblage of activities that leads to the discovery of a new brand particle made of carbon known as (Bucky balls) – sixty carbon atoms organized in a soccer ball shape. In that time period experts felt nothing to study in details.[4]CNTs are allotropes of carbon with different carbon- carbon bonds, discrete physical and chemical belongings with a nanostructures and which could have length - to - diameter ratio superior than one million. Carbon nanotubes bear a similitude of graphite rolled up with hexagonal mesh to form tube like structures and the carbon molecules at the hexagonal apex is also termed as graphene.[5]

In carbon based nanomaterials list, 2D single layer graphite is also identified as graphene. Graphene contains sp3 hybridization which is tougher than the diamond contains sp3 hybridization. Graphene are rolled into tube shape to form carbon nanotubes.[6] CNTs are able to absorb, conjugate and fill the molecules inside the CNT tubes. Scientist evidenced that CNTs were implausible vehicle for drug transport to cells or tissues (target site) without metabolism. CNTs are not only used for drug or gene therapy it is also used for the biosensor diagnosis, tissue regeneration, and enantiomer separation of chiral drugs.[7,8] Chiral meanings they are in tube shape and mostly metallic, the non-chiral nanotubes are separated in to armchair and zigzag. The nanostructures carbon nanotubes (CNTs) have exquisite capability that they employed as systemic transporter of huge number therapeutic compounds. [9,10]

In 1990 multi walled carbon nanotubes were exposed by scientist Iijima formed by arc-discharge method. Carbon nanotubes are segregated as single walled carbon nanotubes and multi walled nanotubes .[11,12] In single walled carbon nanotubes, the carbon atoms attached together by covalent C-C bonds in single graphite sheets having diameter of 0.4 – 3 nm range, In multi walled carbon nanotubes , the distance between two graphite sheets is 0.340 nm, and possessing diameter of 2 to 100 nm and length of 1-50 μm.[13 ,14]

The CNTs are valued devices for antifungal activity, si- RNA , immune therapy, biomedical imaging, biosensor and delivery of anticancer molecules in the treatment of blood cancer, cervical cancer, breast cancer, brain cancer, colon cancer etc.[15]

Cancer demand uneven cellular extension, Through this process, there will be a evolution of a system of new blood capillaries (angiogenesis), activated by diverse signals from carcinoma which include hypoxia, acidic pH, abnormally low blood sugar, anxiety, inflammatory responses and mutations. Cancer divulges several clinical mechanism and drug resistance due to genetic and phenotypic complexity.[16,17,18]

Pristine MWCNTs owning hydrophobic property, the basic exigency for CNTs formulations involved in the drug delivery system is the solubility of CNTs in aqueous solution because it is cardinal for absorption, transportation, secretion. And very important is that CNTs formulations should be uniform and stable. To obtain the fancy CNTs formulations pristine CNTs must be functionalized.

In our learning covalent functionalization is preferred since it will leads to safe add on of functional groups, progresses solubility, and compatibility. Covalent functionalization of MWCNTs was carried out by three methods i) Initial acidic Treatment followed by treatment with Hcl ii) Treatment with Hcl iii) Initial basic Treatment followed by treatment with Hcl, [19, 20, 21, 22,23,24], then followed by non covalent functionalization in order to progress the dispersion of CNTs in liquid medium. The diverse covalent functionalization with acids and non covalent functionalization with PEG polymer are followed. [25,26,27,28]

The foremost intention of work to explore the consequence of drug release from functionalized MWCNTs. Here MWCNTs are endangered to covalent functionalization then it is revised in non covalent functionalization with PEG, that supports accomplish long blood circulation and lessen the intake by reticulo-endothelial system. The attained functionalized

MWCNTs are loaded with 5 Fluorouracil according DOE. F-MWCNTs- 5Fu formulation drug release was statistically analyzed and characterized by SEM, TEM, FT-IR, particle size and zeta potential.

2. Materials and Methods

Materials

Multiwalled carbon nanotube (outer diameter 10- 30 nm, number of walls 5-15, length 1-10μm) was purchased from Nano Wings Private limited, Telangana. 5-Fluorouracil was obtained as gift sample from Nantong Jinghua Pharmaceutical co.,ltd, Jiangsu, China. All chemicals used were of laboratory grade. SEM studies (Model ESEM QUANTA 200) were done at Advanced facility for microscopy and microanalysis, Indian institute of science, Bengaluru. TEM studies (Model-M3000) were done at Centre for Nano Science and Engineering Indian institute of science, Bengaluru. Infrared spectra were recorded at MerieuxNutrisciences Bangalore pvt .ltd. The purity of samples was evaluated via thermo gravimetric analysis (TGA) by using Mettler Toledo analyser at Indian institute of sciences, Bangaluru. Particle size and zeta potential was found by using Malvern zetasizer ZS at Malvern-Aimil application centre, Bengaluru.

3. Design of experiments

Statistical method

The Design of experiment includes three main stages first: the planning phase, the conducting phase, interpretation phase. Taguchi statistical technique is used. It is foremost tool mainly based on orthogonal array designing and optimizing the systemic study. The appropriate Orthogonal array for our learning is selected mainly on process parameter and their levels. According to OA specification experimentation were carried. Experiment results are analyzed by S/N ratio, ANOVA was carried to known the significance and percentage of contribution of each parameter, the combination of process parameters are obtained by the combination of S/N ratio and ANOVA analysis. To verify optimal process parameters the validation experiments were done.

$S/N = -10*\log(\Sigma(1/Y2)/n)$ …… (1)

The equation for higher is better S/N ratio is presented in equation 1

Where y1, y2, y3, ...yn, are the end result of the drug release behavior studied, n is the number of inspections.

Formulations	**Covalent Functionalized MWCNTs**	**Non covalent Functionalized MWCNTs**	**Functionalized CNTs :Drug conc. (5- Fluorouracil) ratio**	**Multi-functionalized MWCNTS: Drug (mg)**
F1	Method 1	PEG	1:1	(50:50)
F2	Method 1	PEG	1:2	(50:100)
F3	Method 1	PEG	1:3	(50:150)
F4	Method 2	PEG	2:1	(50:50)
F5	Method 2	PEG	2:2	(50:100)
F6	Method 2	PEG	2:3	(50:150)

F7	Method 3	PEG	3:1	(50:50)
F8	Method 3	PEG	3:2	(50:100)
F9	Method 3	PEG	3:3	(50:150)

Table 1: Formulation of functionalized MWCNTs loaded with 5-fluorouracil

Formulations prepared by using functionalized MWCNTs and 5- Fluorouracil. [29.30,31,32,33,34]

Covalent functionalization of MWCNTs was carried out by the following three methods.

a) Initial acidic treatment followed by treatment with Hydrochloric acid.

b) Treatment with conc. Hydrochloric acid.

c) Initial basic treatment followed by treatment with Hydrochloric acid.

Subsequently, the following procedures were done to attain non covalent functionalized MWCNTs. Covalent functionalized MWCNTs gained by the above three methods are once again functionalized by using PEG, PEG-functionalized MWCNTs was prepared by taking 500mg of covalent functionalized carbon nanotubes were dispersed in 100 ml PEG solution (1gm in 10 ml) subject to sonication by fast clean ultra sonic bath sonicator for 15mins. Unbound PEG was separated by centrifugation process at speed of 7000 RPM / 5min. Supernatant was discarded and the precipitate is filtered through 0.2 micro filters through vacuum filter and dried in room temperature. Wholly weighed functionalized MWCNTs were dispersed in drug solution (5-Fluorouracil in methanol {1 mg / ml}) at varied concentrations as depicts in table 1, sonicate the dispersion for 10 to 15 mins. Fill the dispersion into plastic tubes tighten with cap, fix it on rotor clamp, the dispersion is rotated for 24 h with a speed of 10 rpm/min, in order to facilitate the loading of drug on MWCNTs. Subsequently, the mixture was exposed to centrifugation process for 10 minutes at 3000 RPM/ 10mins, the precipitate was washed with methanol and again washed three times with pure water and centrifuged to get rid of unbound drug. The solid samples dried at 30 ^{o}C by applying the vacuum for 24 h to obtain F- MWCNTs- 5Fu. All samples were stored in vacuum desiccators at room temperature.

Scanning Electron Microscopy Analysis

SEM studies (Model ESEM QUANTA 200) were established on the basis of irradiation of the MWCNTs sample by an electron source. Take a small droplet of sample on SEM grid, the sample essential be in dry condition so evaporate the solvent, and observed in gaseous environment in vacuum chamber at pressure of -1×E-3 Pa. An electron beam is scrutinized across the sample and back scattered electron are spotted to harvest an image of sample morphology.

Transmission Electron Microscopy Analysis

To study the structural characteristics of MWCNTs samples TEM studies were accomplished. Prepare the MWCNTs sample by consuming Ethanol, place the 5 drops of sample on TEM copper grid, take away the excess solvent present on the grid by means of filter paper, then dry under infrared light and scan in the TEM instrument.

FT-IR studies

Take the sample holder, cleaned by using acetone and dry with Kim wipes. The sample pellet is prepared by using 100 mg of potassium bromide and 5 mg of MWCNTs conjugate, the pellet is positioned in the sample holder. In scan range limit 4000-400 cm^{-1} the samples were documented, detected the pressure applied, fine tuned the resolution to 4, and lastly scrutinized the IR spectra.

Thermo gravimetric Analysis

TGA is the modest method which accords the data by a simple thermo gram, take 1-20 mg of sample in aluminium oxide crucible, keep the sample loaded crucible on auto sampler board, which chairs them in the furnace. In STAR e software give sample name, adjust heating temperature: RT to1000, heating rate: 10k/min, purge gas:N2, then start experiment.

Measurement of Particle size

Particle Size was examined by Malvern zetasizer ZS at Malvern-Aimil application centre, Bengaluru. Partricle size determination finished by dynamic scattering method. MWCNTs sample was briskly shaken to aggregate. Draw 1 ml sample in transparent throwaway cuvette.

Measurement of Zeta Potential

Zeta Potential was evaluated by Malvern zetasizer ZS at Malvern-Aimil application centre, Bengaluru. The firmness of colloidal dispersion was measured by zeta potential. Sonicate the MWCNTs sample for 15 mins at 90 Hz, take one ml sonicated sample in zeta potential cuvette, then measure the zeta potential.

Drug Entrapment Efficiency

Take 50 mg of all nine prepared formulations in distinctly in 150 ml tarson tubes and add 100 ml of phosphate buffer pH 7.4, immerse the tubes in water bath maintained at 37 ˚C for 1 h. centrifuge at 10,000 r/min to remove the drug which entrapped in MWCNTs, separate the supernatant liquid, take 1 ml aliquot and dilute suitably to determine the amount of drug loaded to MWCNTs by using UV spectrophotometer at 266 nm.

Statistical analysis of *In- vitro* Drug Release Studies

In-vitro release studies are carried out for all nine formulations in different pH conditions, as designed in DOE. To swot the drug release pattern take dialysis membrane -70 (HIMEDIA) in length of 1.2 inch tube, tie the ends of tube to form a pouch shape and prepare phosphate buffers pH 6.3, 7.4, and 8.0 and were used as dissolution medium. Take 25 mg of all formulations soak in dissolution medium for overnight according to table 2. Fill the soaked drug solutions in to tubes formed by dialysis membrane. Immerse in 100 ml of phosphate buffer of same pH of the tube in different conical flask maintained at a temperature of 37 ˚C on shaking water bath. Take 1 ml volume of aliquots at regular intervals, dilute suitably with buffer and analyzed by UV-Vis spectrophotometer at 266 nm. Measured results attained were analyzed by MINITAB software.

4. Results and discussion

SEM studies

SEM studies were done at Indian institute of science, Bangalore. Functionalized MWCNTs of SEM emblematic images are given in figure 1a, 1b, 1c, and 1d taken at 200 nm, 500nm, 1 μm , 2 μm respectively. SEM illustrative images of F-MWCNTs-5Fu are stated in figure no

2a, 2b, 2c, 2d taken at 200 nm, 500nm, 1 μm, 2 μm respectively. The tube structures of the functionalized MWCNTs and F-MWCNTs-5Fu are fairly diverse from Pristine MWCNTs. The increase in the tube diameter size designates that the MWCNTs are functionalized and drug loaded firmly in to functionalized MWCNTs. In SEM images overlying are perceived, which might be as a repercussion of aggregation in dry condition.

TEM studies

TEM studies were done at Indian institute of science, Bangalore. TEM study divulges the internal structure of drug loaded Functionalized MWCNTs. TEM images F-MWCNTs-5Fu are portrayed in 3a, 3b, 3c, and 3d figures and taken at 100nm, 200 nm, 500nm, 1 μm respectively. TEM images evidently exemplifies that the drug molecules are conjugated with the MWCNTs. Typically, the outsized tube diameters displays 5-Fluorouracil molecules are placed favorably in tubes with a larger inner diameter.

FT-IR studies

The functionalized MWCNTs were characterized by FT-IR spectroscopy recorded in a range of 4000 to 400 cm-1 (Perkin Elmer Spectrum II) done at Merieux Nutrisciences Bangalore pvt .ltd. The FT-IR spectra for Functionalized MWCNTs were showed in figure 4a. The peaks for carboxy group at 1651.71 cm^{-1} and hydroxyl group at 2340.02 cm^{-1} (range 3300- 2500 cm^{-1}) for covalent functionalization, the strong absorption spectra analysis of PEG400 are assigned to -CH2CH2- stretching around 2886 cm^{-1}, the presence of saturated carbons (CH2CH2)n established at 3749 cm^{-1}. The FT-IR spectra of F- MWCNTs – Fu exemplify in figure 4b. The peaks at 1655.63cm^{-1}, 1349.35cm^{-1}, 3120.65cm^{-1} and 1246.19cm^{-1} perceived in the graph signposts the presence of C=O, C=C, N-H, and C-N stretching vibrations agreeing to 5-Fluorouracil, and conspicuous for the absorption bands of Functionalized -MWCNTs- 5-Fluorouracil complex. The peak at 1349.35 cm^{-1} denotes to vibration of pyrimidine compound approving 5-Fluorouracil. FT-IR measurements were carry out to present the indication of attachment of PEG polymer and 5 Fluorouracil to MWCNTs.

Particle size

The particle size of the Functionalized MWCNTs, Functionalized –MWCNTs - 5-Fluorouracil conjugate showed in figure 5a , 5b and zeta potential of the Functionalized MWCNTs, Functionalized –MWCNTs - 5-Fluorouracil conjugate showed in figure 6a, 6b were characterized by a laser particle size analyzer at Aimil limited, Bengaluru. The freeze dried samples exhibited a mean particle size of Functionalized MWCNTs and F- MWCNTs - 5Fu is 233 (d.nm) with 0.256 PDI and 378 (d.nm) with 0.591 PDI respectively. The zeta potential of Functionalized MWCNTs and Covalent F-MWCNTs- 5-Fu conjugate was found to be -6.21 mV, -2.47 mV respectively.

TGA

Thermal stability of Functionalized MWCNTs and F- MWCNTs - 5Fu were established in figure 7a and 7b using a thermo gravimetric analyzer at Indian institute of sciences, Bangaluru. Thermo gram curve generated as percentage weight loss VS temperature and time. MWCNTs samples were heated from 0 to 1000 ˚C. The burning of sample start themselves, due to the presence of amorphous carbon in MWCNTs, samples initial burning temperature was take place continues up to 1000 ˚C and the curve shows the residue left in the TGA furnace. The residue left in the furnace due to the drug found in MWCNTs.

Drug entrapment efficiency

Drug entrapment efficiency of all nine formulations was done by analyzing the drug concentration in UV- Vis spectrophotometer. Figure 8 exemplifies the Stacked UV graph of drug concentration of all nine formulations. In our study maximum drug entrapment was 86.28%, Entrapment for the formulations F1, F2, F3, F4, F5, F6, F7, F8 and F9 shows realistically good percentage of entrapment efficiency with 65.60%, 69.95%, 60.29%, 68.60%, 66.96%, 72.56%, 85.12%, 83.77% and 86.28% respectively.

Statistical analysis of in vitro drug release

In our present study statistical analysis done by using MINITAB software, experiment conducted on L18 orthogonal array, and experimental parameters are (i) Drug release time (ii) Covalent and Non covalent functionalized CNT (iii) Drug concentration (iv) Buffers. In our study is drug release is response, As per Taguchi L18 array the experimental details and levels tabulated in table 1. Total 18 tests and all parameters are varied for 6, 3, 3 and 3 levels respectively. The results of drug release and the S/N ratio for time obtained in each experiment is showed in table 2. By using signal to noise ratio, the influence of process parameters such as drug release, time, functionalized CNTs, drug and buffers were analyzed. The values in experiments no 15, 16 results are apparently optimal. It is clearly determined that F3 and F7 formulations display superior percentage of drug release in buffer pH 7.4 at 52 hours among nine formulations. In table 3 the different combination of parameters for analysis of variance was tabulated. ANOVA table enlightens factors which are momentous on drug release based p- value. This study model swift given in table 4, it gives R^2 value 93.1% measures the model degree of fitness, which elucidate accord that, our study model clarify the association between input factors and response variables. Response table for S/N ratio presented in table 5. The rank values presents the influence level of input factors on response variables. To maximum extent time parameter influences the drug release, it is also affected by the effect functionalized CNTs, Buffers, and drug ratio. Figure 9 explains main effect plot for S/N ratio, it clarify that when all the factors increases the S/N ratio will also increases, S/N ratio is maximum is desirable, but the better option is high S/N ratio. Residual plots for drug release given in figure 10. No evidence of any pattern in plots of residual verses order values, so constant variance assumption is appreciative. Our experimental data meets the normality assumption because the normal probability plot explains that the line dividing the points equally into two half. In standardized residual verses the fits plot, the residual scattered randomly to zero. There is no proof of non-constant variance, missing terms and unusual structures, so we conclude that experimental data satisfies the independent/ randomization assumption.

a) SEM studies:

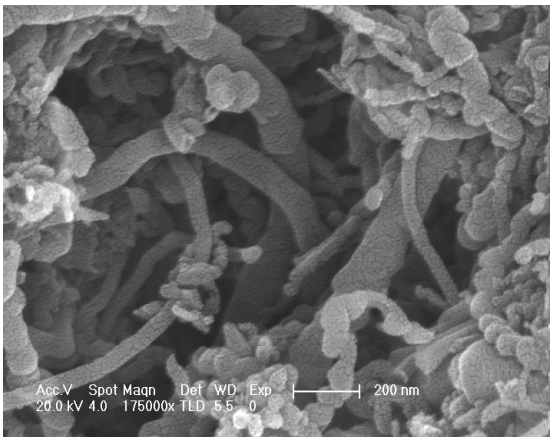


Figure 1a: SEM images of Functionalized MWCNTs at 200 nm

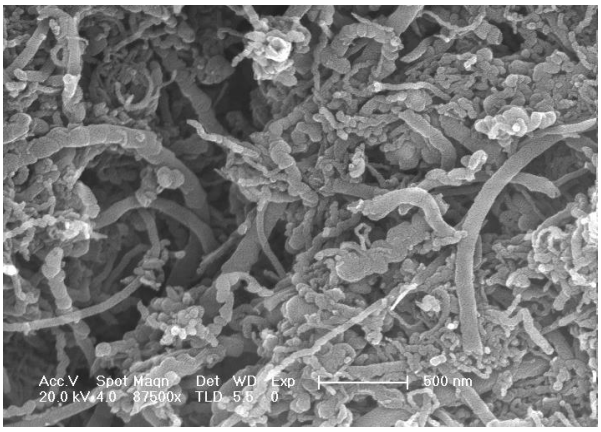


Figure 1b: SEM images of Functionalized MWCNTs at 500 nm

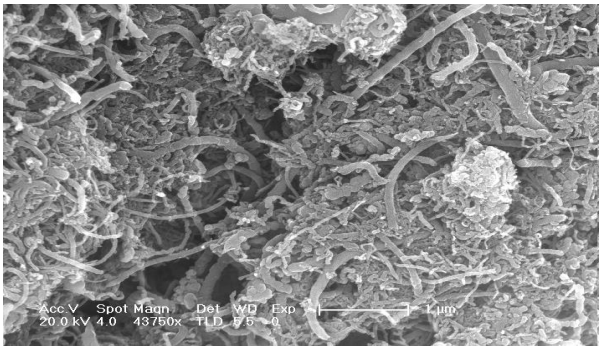


Figure 1c: SEM images of Functionalized MWCNTs at 1µm

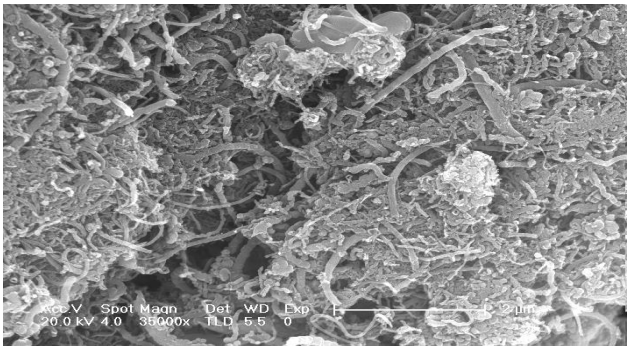


Figure 1d: SEM images of Functionalized MWCNTs at 2µm

SEM images of F- MWCNTs – 5-Fu:

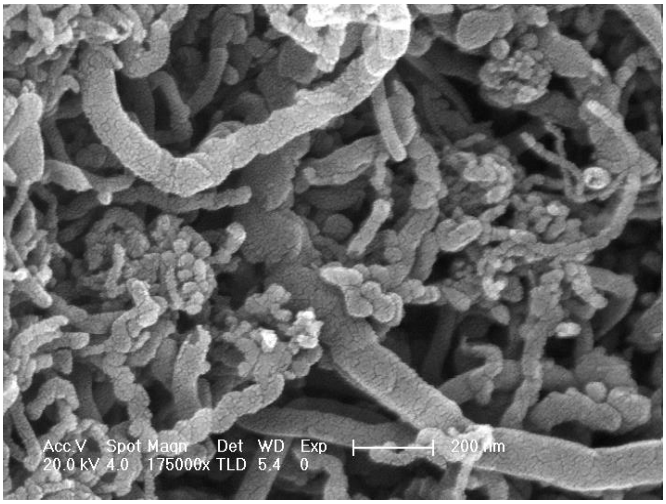
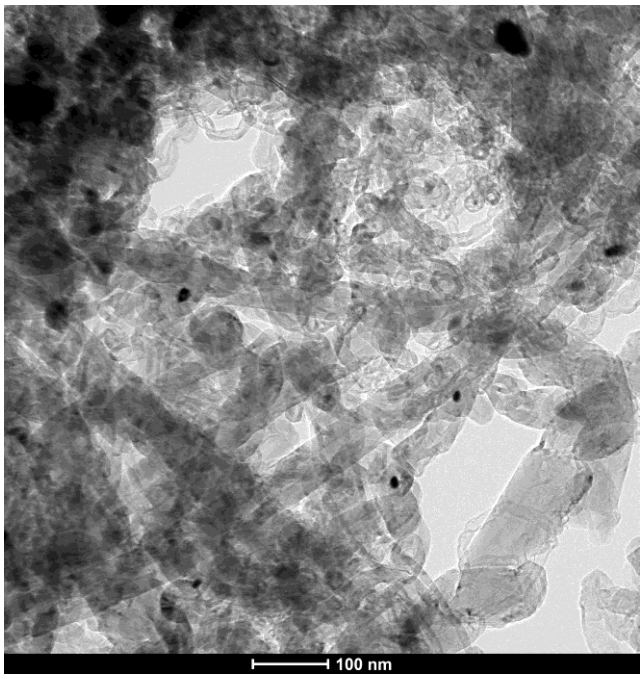
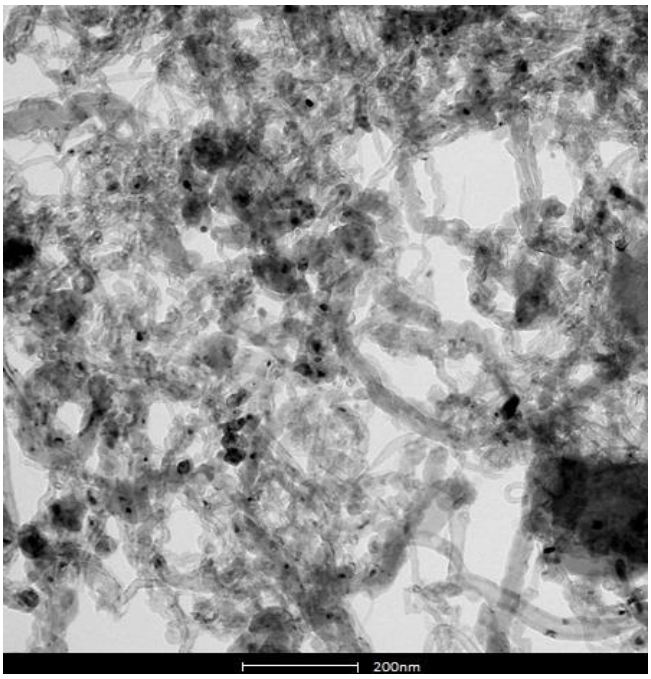


Figure 2a: SEM images of F- MWCNTs – 5-Fu at 200nm

Figure 2b: SEM images of F- MWCNTs – 5-Fu at 500nm





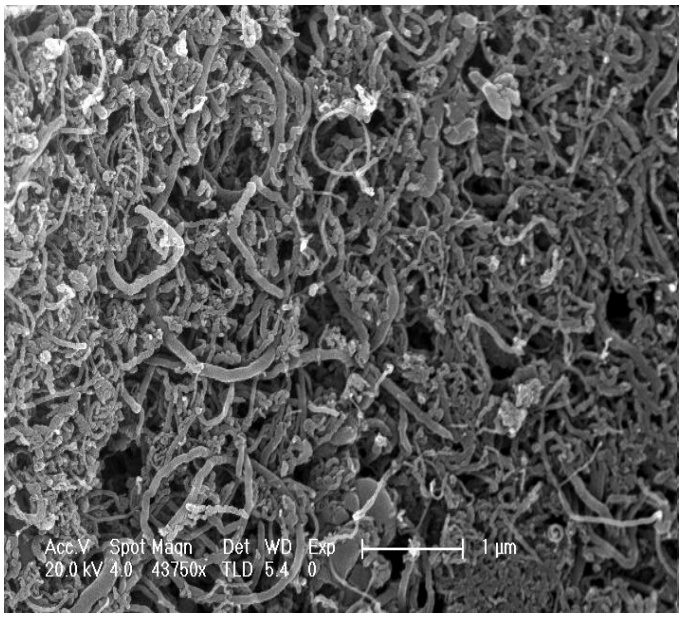


Figure 2c: SEM images of F- MWCNTs – 5-Fu at 1 µm

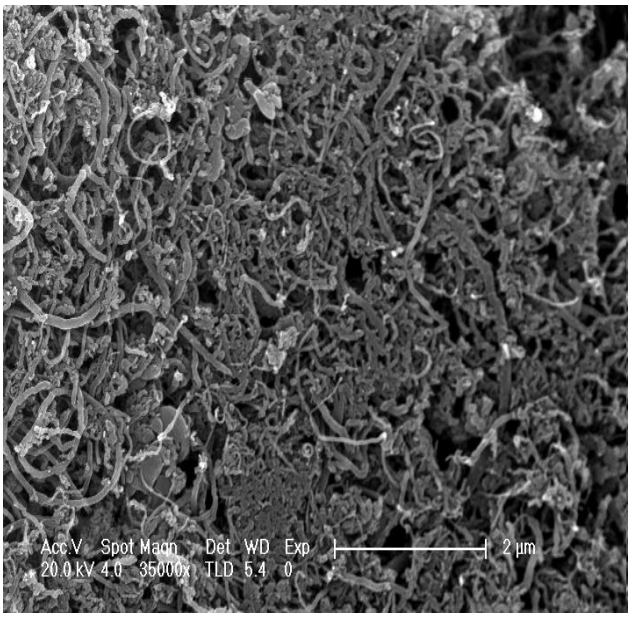


Figure 2d: SEM images of F- MWCNTs – 5-Fu at 2 µm

b) TEM studies:

Figure 3a: TEM images of F- MWCNTs – 5-Fu 100 nm

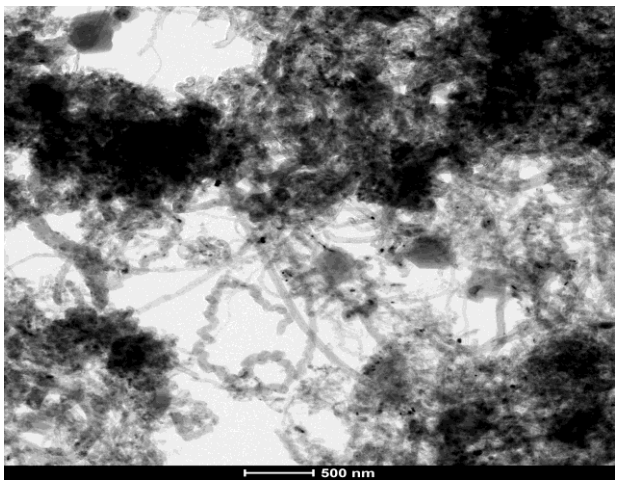


Figure 3b: TEM images of F- MWCNTs – 5-Fuat 200 nm

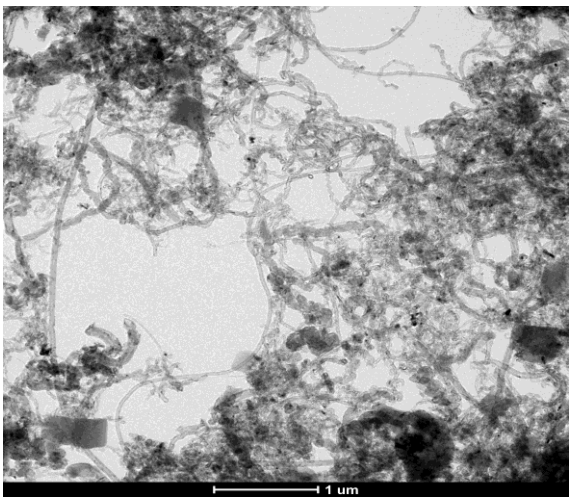


Figure 3c :TEM images of F- MWCNTs – 5-Fu at 500 nm

Figure 3d TEM images of F- MWCNTs – 5-Fu at 1 µm

c) FTIR studies :

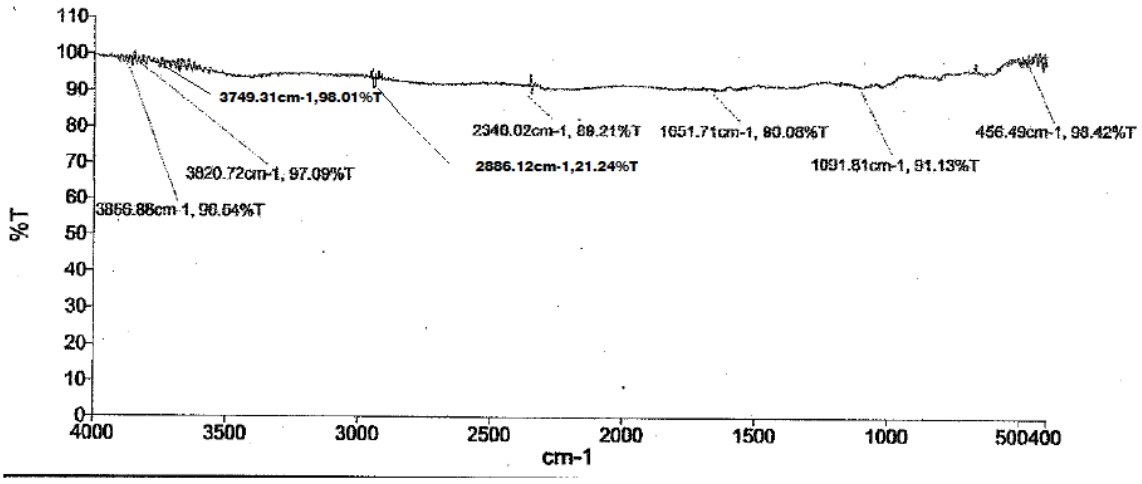


Figure 4a: FT-IR graph of Functionalized MWCNTs

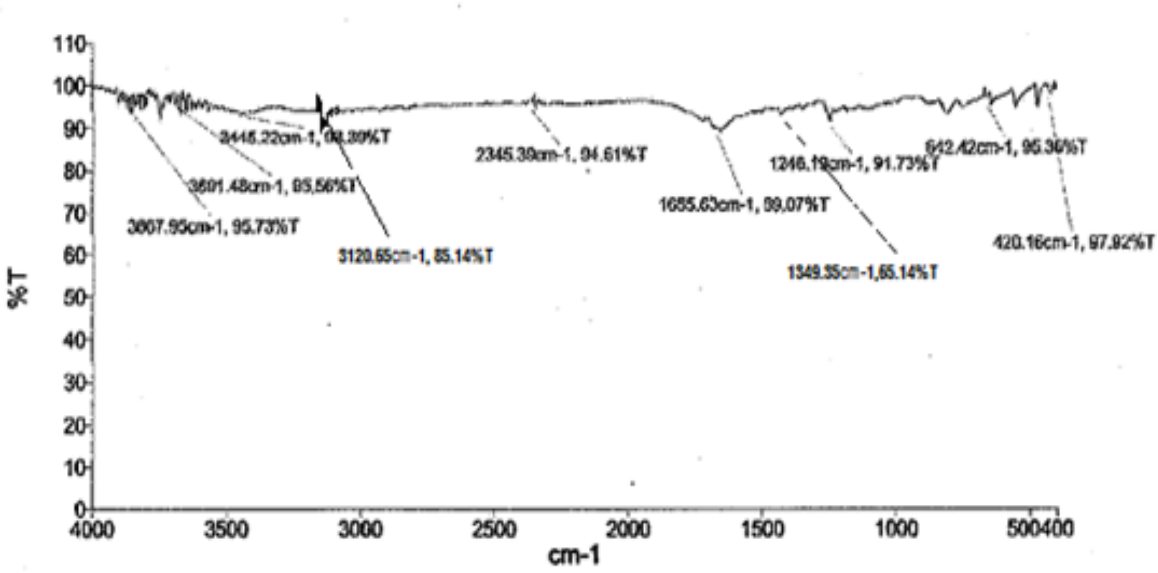


Figure 4b: FT-IR graph of F- MWCNTs – 5-Fu.

Particle size :

Results

			Size (d.nm...	% Intensity:	St Dev (d.n...
Z-Average (d.nm):	233.4	Peak 1:	280.2	95.7	148.1
PdI:	0.256	Peak 2:	4368	4.3	937.3
Intercept:	0.867	Peak 3:	0.000	0.0	0.000
Result quality	Good				

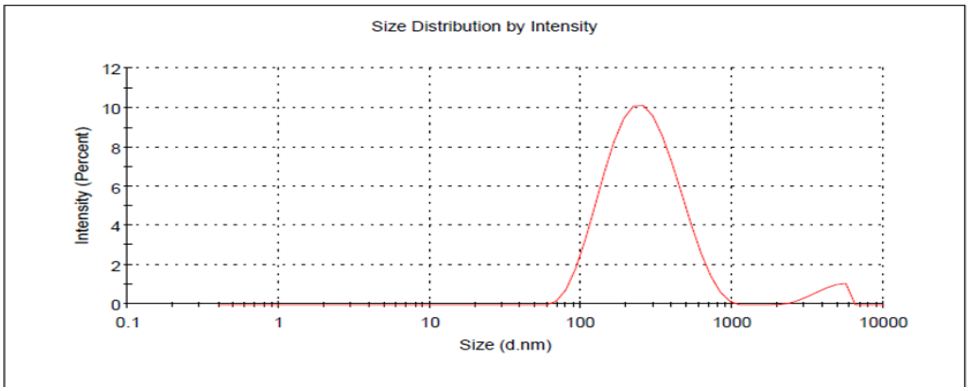


Figure 5a:Particle size of Functionalized MWCNTs

Results

			Size (d.nm...	% Intensity:	St Dev (d.n...
Z-Average (d.nm):	378.2	Peak 1:	1011	51.3	626.7
PdI:	0.509	Peak 2:	233.7	41.5	95.45
Intercept:	0.783	Peak 3:	4363	6.9	876.4
Result quality	Good				

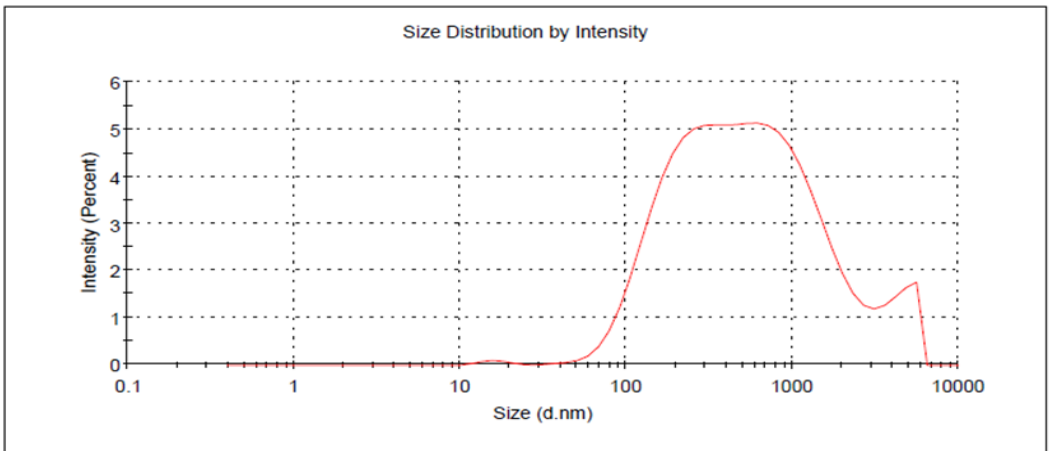


Figure 5b: Particle size of F- MWCNTs – 5-Fu

Zeta potential :

Results

			Mean (mV)	Area (%)	St Dev (mV)
Zeta Potential (mV):	-6.21	Peak 1:	-6.21	100.0	3.69
Zeta Deviation (mV):	3.69	Peak 2:	0.00	0.0	0.00
Conductivity (mS/cm):	1.07	Peak 3:	0.00	0.0	0.00
Result quality	Good				

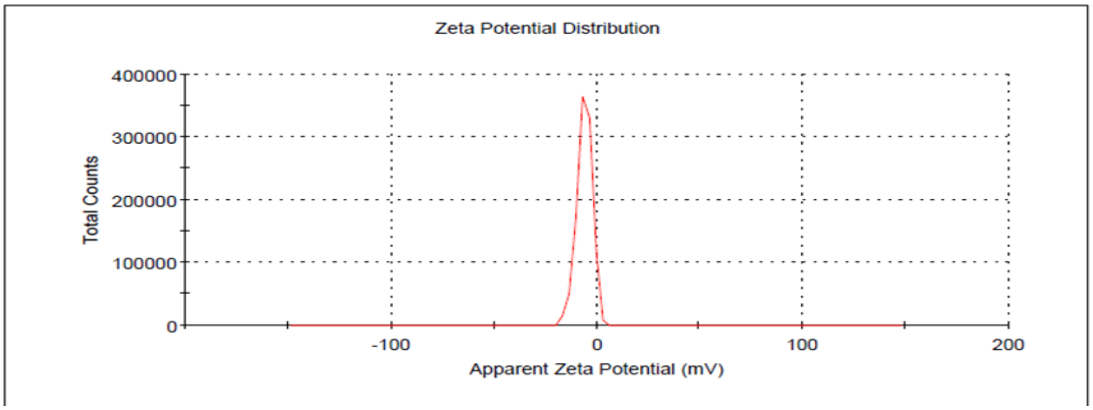


Figure 6a: Zeta potential of Functionalized MWCNTs

Results

		Mean (mV)	Area (%)	St Dev (mV)
Zeta Potential (mV): -2.47	Peak 1:	-2.47	100.0	3.86
Zeta Deviation (mV): 3.86	Peak 2:	0.00	0.0	0.00
Conductivity (mS/cm): 1.07	Peak 3:	0.00	0.0	0.00
Result quality Good				

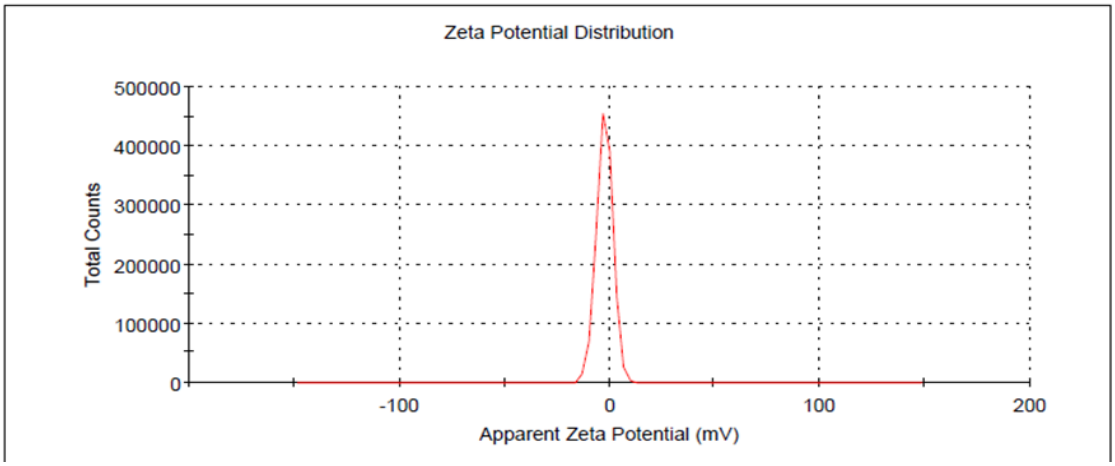
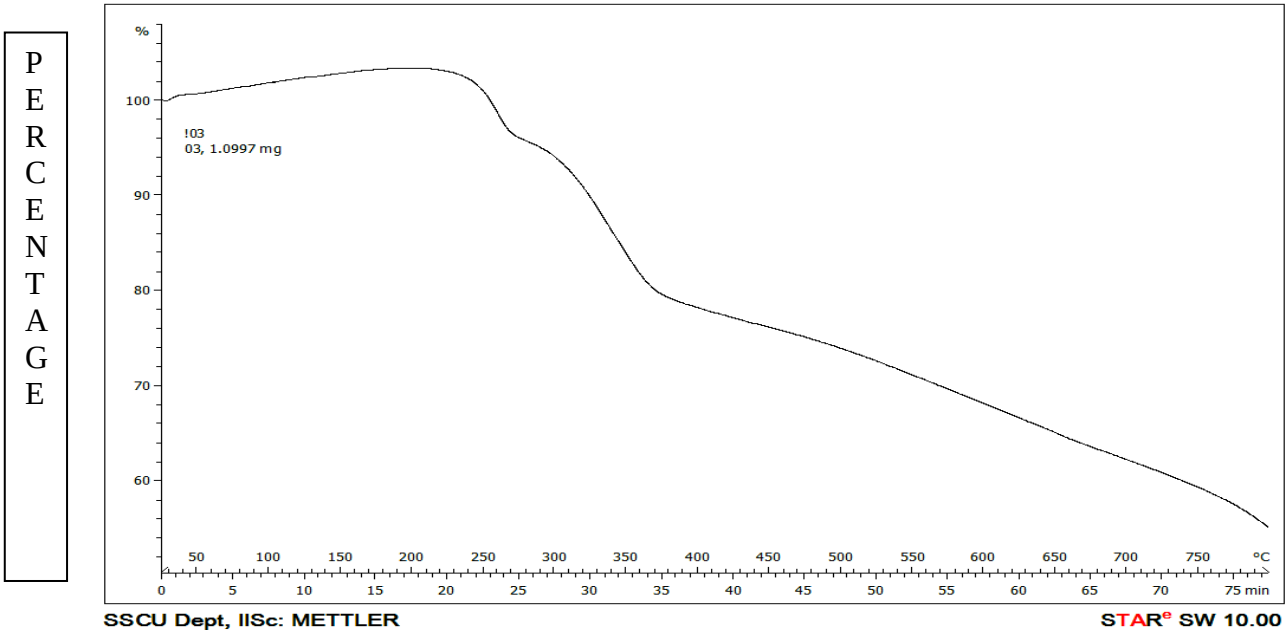


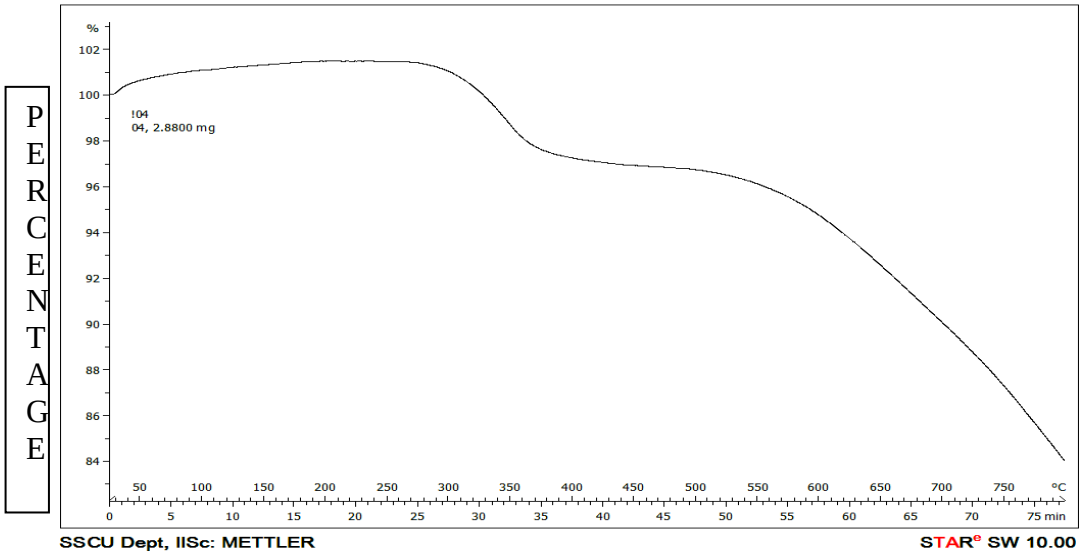
Figure 6b: Zeta potential of F- MWCNTs – 5-Fu

Thermo gravimetric analysis



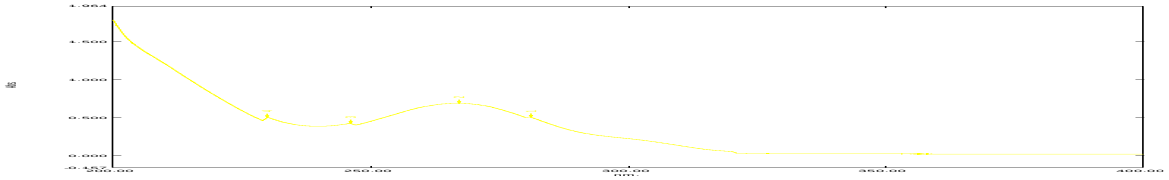
Temperature (in Degrees) and Time (in Minutes)

Figure 7a: TGA analysis of F- MWCNTs

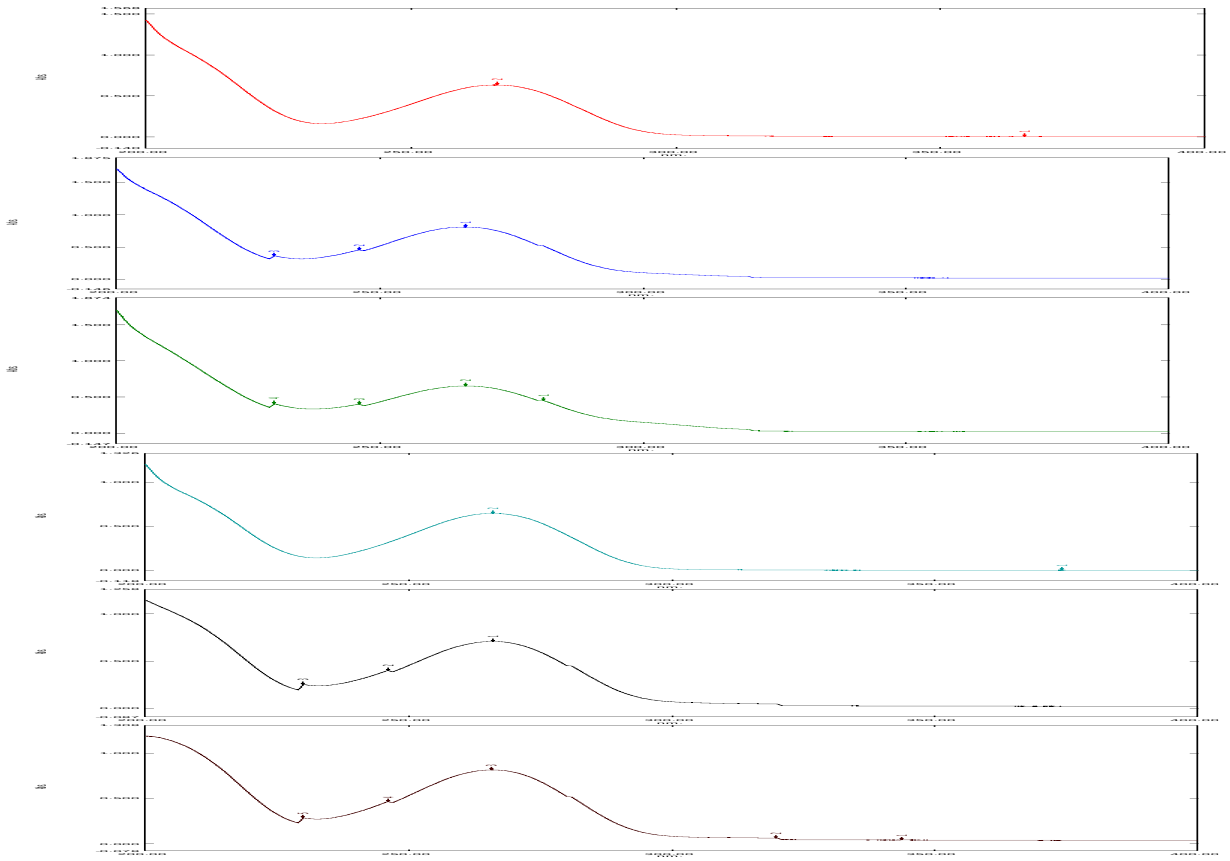


Temperature (in Degrees) and Time (in Minutes)

Figure 7b: TGA analysis of F- MWCNTs – 5-Fu



Drug entrapment Efficiency :



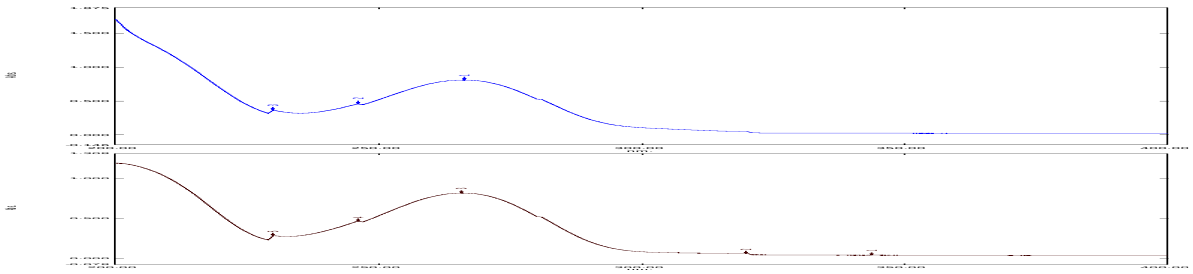


Figure 8: Stacked UV graphs of all 9 formulations

h) Drug *in-vitro* release studies:

SL. NO	Drug release time (hr)	Covalent functionalized MWCNTs (Methods)	Non-covalent functionalized MWCNTs	Drug conc (ratio)	Buffers (pH)	Drug release (%)	Signal to noise ratio analyzed for the first time
1	4	1	1	1	6.3	5	13.9794
2	4	2	2	2	7.4	8	18.0618
3	4	3	3	3	8.0	9	19.0849
4	12	1	1	1	7.4	14	22.9226
5	12	2	2	2	8.0	19	25.5751
6	12	3	3	3	6.3	21	26.4444
7	22	1	1	2	6.3	21	26.4444
8	22	2	2	3	7.4	35	30.8814
9	22	3	3	1	8.0	36	31.1261
10	32	1	1	3	8.0	34	30.6296
11	32	2	2	1	6.3	37	31.3640
12	32	3	3	2	7.4	45	33.0643
13	42	1	1	2	8.0	71	37.0252
14	42	2	2	3	6.3	75	37.5012
15	42	3	3	1	7.4	81	38.1697
16	52	1	1	3	7.4	82	38.2763
17	52	2	2	1	8.0	75	37.5012
18	52	3	3	2	6.3	80	38.0618

Table 2: Result of orthogonal array of Taguchi for Drug Release (Signal to Noise ratio Analyzed for first time)

SERIAL NO	Source	DF	Adj SS	Adj MS	F-value	P-value
1	Drug release time	1	12466.3	12466.3	174.27	0.000
2	Functionalized CNT	1	168.7	168.7	2.36	0.149
3	Drug ratio	1	5.3	5.3	0.07	0.789
4	Buffers	1	2.1	2.1	0.03	0.867
5	Error	13	930.0	71.5		
6	Total	17	13572.4			

Table 3: Analysis of variance

R-sq R-	sq(adj)
0.931	0.910

Table 4: Model summary

Level	S/N ratio of TIME	S/N ratio of CNT	S/N ratio of DRUG	S/N ratio of BUFFER
1	7.333	37.833	41.333	39.833
2	18.000	41.500	40.667	44.167
3	30.667	45.333	42.667	40.667
4	38.667	---	---	---
5	75.667	---	---	---
6	79.000	---	---	---
Delta	71.667	7.500	2.000	4.333
Rank	1	2	4	3

Table 5 Response Table for Signal to Noise Ratios

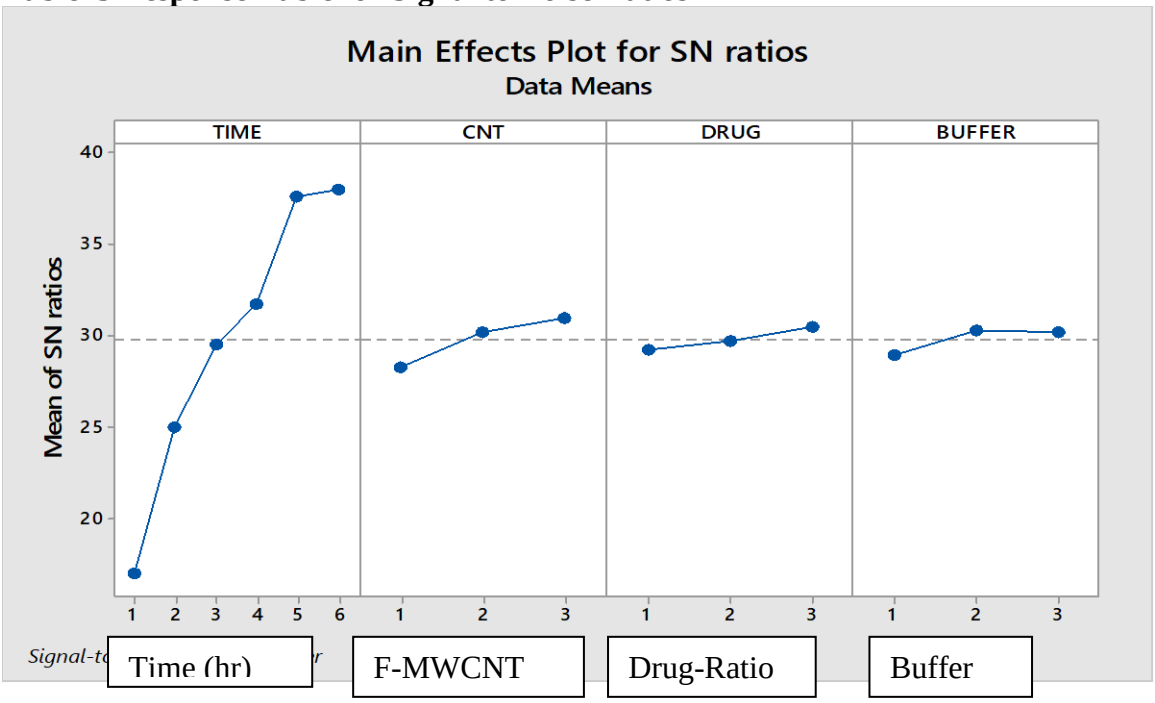


Figure 9: Main effects plot for means

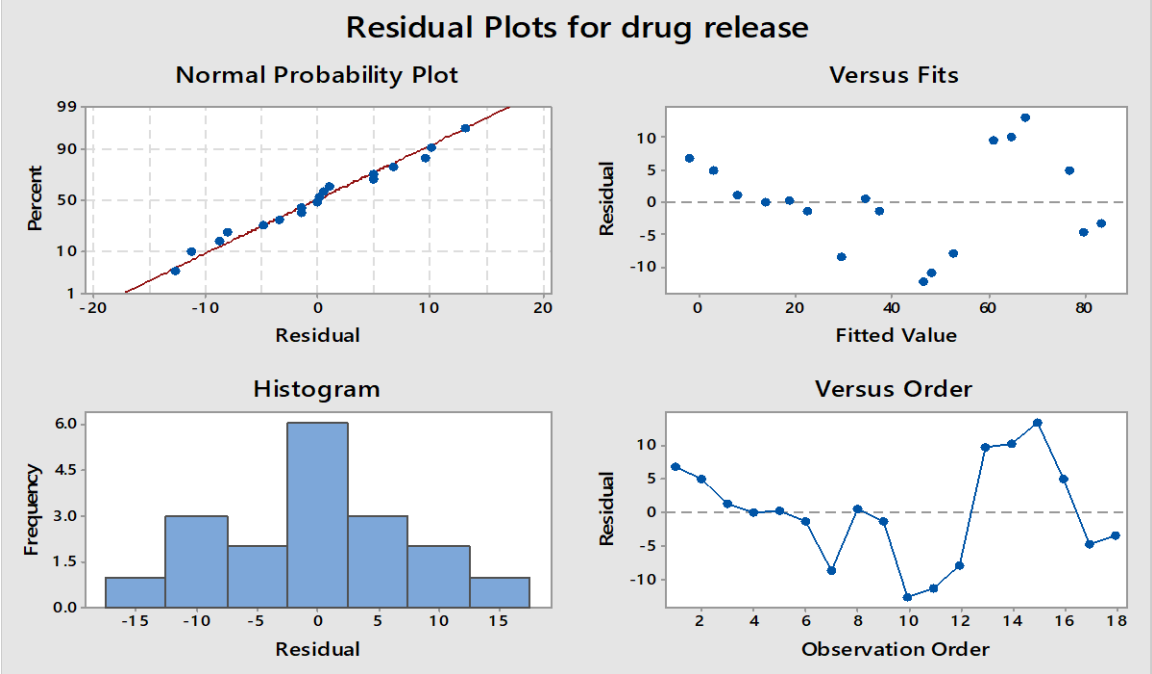


Figure 10: Residual plots for drug release

5. Conclusion

In our research work we have successful prepared the controlled release formulation of Functionalized MWCNTs 5 -Fluorouracil complex. Covalent and non covalent Functionalization was executed for pristine MWCNTs. Preceding MWCNTs were functionalized by covalent functionalization by means of three methods followed by Non covalent functionalization using PEG polymer, PEGlyation improves the physical conjugation of drug to MWCNTs and aid to release in controlled manner. Functionalized MWCNTs 5 -Fluorouracil complex was prepared by simple method using functionalized MWCNTs, drug loading can be studied by morphological studies such as SEM and TEM, which clearly authorizes that MWCNTs internal and external diameter and size has been expanded. FT-IR studies of the MWCNTs conjugate sanctions the occurrence of functional group of COOH group and PEG, and it also indicates the 5Fu loaded to F- MWCNTs. This is further confirmed by TGA analysis. Drug loading onto the functionalized MWCNTs in all formulation displays upright percentage of drug entrapment with minimum 60% to 83 %. An increase in particle size found when functionalized with PEG was about 233 (d.nm), further increase in particle size of formulation was about 378 (d.nm) when drug loaded with F-MWCNTs. The Zeta potential of F- MWCNTs was -6.21 mV and the formulation F-MWCNTs- 5-Fu was -2.47mV, and found be negative. The release of 5- Fluorouracil from F-MWCNTs- 5-Fu formulation was governed by phosphate buffer at various levels of pH in the release medium. The drug released in a controlled manner with a prolonged release property. The data obtained analyzed statistical MINITAB software.

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Conflict of interest

The authors own no conflict of interest to pronounce.

Ethical approval

This research is under progress of animal study.

Authors contributions

All authors contributed toward data analysis, drafting, or critical revision of the paper, and agree to be accountable for all aspects of the work. All authors have revised and agreed the final draft and are accountable for the content and resemblance index of the manuscript.

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