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PREPARATION, CHARACTERISATION AND IN-VITRO RELEASE OF ANTI-CANCER ACTIVITY OF MWCNTS FUNCTIONALIZED WITH CARBOXYLIC GROUP

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ABSTRACT

Functionalized carbon nanotubes developed by different functionalization processes, Functionalized CNTs possess the versatileness to act as composite to deliver the anticancer drugs. Viable effects and release of anticancer drug by preparing new neoplasia targeting drug delivery system by loading Irinotecan to MWCNTs that can implement essential challenges in the cancer treatment. Pristine MWCNTs are subjected to covalent functionalization by different methods to obtain consistent and steady dispersion of MWCNTs. Irinotecan complexed with functionalized MWCNTs evaluated by different methods such as SEM, FT-IR, TEM, TGA, Particle size analysis, Zeta potential, Drug entrapment efficiency, and Drug release studies. SEM AND TEM studies shows drug was well complexed to the MWCNTs. TGA results shows the Purity of MWCNTs, *in vitro* drug release analysed by statistical method, preparation of MWCNTs-Irinotecan complex is easy, commodious and come up with better development. Neoplasia drug containing Irinotecan may improve drug release and lessen the side effects.

KEYWORDS: MWCNTs, Neoplasia, Nanoparticles, ANOVA. Taguchi method, Orthogonal Array.

INTRODUCTION

Nanotechnology is a platform for neoplasia treatment and in many healthcare applications such as advancement in nanomedicines, diagnostics, and drug delivery. In cancer treatment, the drug delivery is very powerful to progress the effects of drug and to lessen the toxic side effects. Nanotechnology is the generation and application of materials in nanometre scale and controls from the level of atoms, molecules, and supramolecular design.[1,2,3]

Neoplasm is atypical, unrestrained growth of new tissue and division of cells, which have the capacity to permeate, attack, and enlarge to the other parts of the human body, universally in every year cancer leads to second major cause of death. World Health Organization approximated, in upcoming cancer deaths will increases. The present neoplasia treatments are radiation therapy, chemotherapy, surgery or amalgamation of all these treatments.

Chemotherapy drugs acts first and leading by hindering the DNA synthesis and mitosis, it leads to death of cancer cells which are growing and dividing rapidly and also degeneration of normal cells and tissues. Chemotherapy drugs harvests serious nasty adverse effects on tissues and organs.[4]

Nanonature carbons/ carbon allotopes covers huge types of carbon structures, such as carbon fibres, fullerene, diamond shape carbon, Carbon nanotubes, graphenes and graphite. Astonishing significance has given to Carbon nanotubes and grahene, as they contribute pointedly in current nanomaterial advances.[5] Among carbon allotropes the graphite is most recurrent. Graphene is a materfamilias of carbon, graphene sheets are enfolded to form 0D fullerenes, 1D nanotubes designed by twisting or stacked to scheme 3D graphite. As they planned and own different modes of activities as investigators, biological imaging, and in the delivery of therapeutic substances including DNA and RNA, proteins, and drugs.[6,7]

Carbon nanotubes are cyclindrical tools with excellent structural, mechanical, and electronic properties. Carbon nanotubes are classified into two types: Single walled carbon nanotubes, and Multi walled carbon nanotube. Sumio Iijima was the first scientist recognized multi layered carbon nanotubes(MWCNTs) in 1991, with a diameter up to 100 nm and the space amid adjoining layer was around 0.34 nm and produced by carbon arc discharge, laser ablation, chemical vapour deposition, and flame synthesis method.[8, 9, 10, 11, 12,13]

In our study we have chosen MWCNTs for cancer drug delivery system instead of SWCNTs, because the less leakage of encapsulated drug from the MWCNTs, leakage may affect measurement of drug release from the side walls of the tube.

Commercially produced CNTs are gravely contaminated with metallic impurities and amorphous carbon, and generally acknowledged as insoluble, less biocompatible, and leads to the formation of clusters. Cluster formation makes CNTs apathetic to chemical treatment. CNTs purification process is strenuous, thus a number of techniques have been outlined to add on functional group to CNTs termed as functionalization. CNTs dispersion should be always consistent and stable for the delivery of drugs, to diffuse the CNTs in liquid medium four fundamental approaches have been used obtain a uniform dispersion: a) Surfactant – assisted dispersion b) Solvent dispersion c) functionalization of CNTs d) Biomolecular dispersion [14]. The CNTs exceptional high surface to volume ratio property permits the loading of different molecules per unit geometric area. Scientists have divided the functionalization of CNTs into two types: a) Covalent functionalization b) Non-covalent functionalization.[15 ,16,17,18,19,20]

HCl is a non-oxidative acid and cannot introduce oxygen-containing groups to CNT wall, but it can enhance the exposure of amorphous carbon. The exposure of amorphous carbon will lead to MWCNT with very low amount of graphitic nanoparticles, bearing higher oxygen existences. The presence of oxygen-containing groups facilitates the exfoliation of CNT bundles, and increases the solubility in polar media. HCl-treated MWCNT provided more available reaction sites, leading to enhanced sidewall functionalization. Strong acid used is nitric acid (HNO_3), or a mixture of Sulphuric acid (H_2SO_4) and Nitric acid (HNO_3). Each study of strong acids functionalization successfully produces carboxylic groups on the surface of the CNT. However, the results are not maximized. Other results showed that CNT produced yet stable and perfectly dispersed. This study is to obtain the effect of adding HCl to the mixture of H_2SO_4-HNO_3 which expected to increase the dispersibility of functionalized CNT. The previous literature studies of CNT functionalization have dispersion time less than 20 days. This study will improve the time required for CNT to settle again which demonstrates the CNT's dispersion capabilities. By the high dispersibility, CNT will be easier to circulate, not easily settle, and last longer in blood vessel without causing the blockage.[21-25] Functionalization of these MWCNTs may increase biocompatibility, append ability of the drug to CNTs, and permeability of drug to cells and decrease the cytotoxicity of the CNTs.[26]

In our study covalent functionalization is chosen, covalent functionalization is regularly carried out by the oxidation of carbon nanotubes by refluxing with strong acids, strong acids enhance the CNTs properties by introducing chemical functional group on the sidewalls of the CNTs leads to the formation of carboxylated CNTs. Functionalized MWCNTs equip with extra spare sites leads to multiple functionalization, enhances the CNTs dispersion, In this study, MWCNTs were first functionalized with acids, successfully loaded with Irinotecan, then the complex were evaluated. These findings have demonstrated that Irinotecan -MWCNTs have a potentially important role in the fast and prolonged release.

MATERIAL AND METHODS

Multiwalled carbon nanotube (outer diameter 10- 30 nm, number of walls 5-15, length 1- 10μm) was purchased from Nano Wings Private limited, Telangana. Irinotecan was obtained as gift sample from Nantong Jinghua Pharmaceutical co.,ltd, Jiangsu, China. All chemicals used were of laboratory grade.

1. DESIGN OF EXPERIMENTS

Statistical technique

Taguchi technique is a robust statistical method provides well organized access to optimize the experiment patterns to conduct, and based on quality and price. Design of experiments (DOE) consists three phases: planning phase, conducting phase, and interpretation phase. Analysis of variance is carried out to detect the parameter which is compelling over the other and observe the percentage of contribution on each drug release parameters. Orthogonal array selected should be acceptable for the experiments according to OA specifications. The results were scrutinized by signal to noise ratio (S/N) values. The S/N ratios are a) Lower the better b) Higher the better c) Nominal the best. The Mathematical equation for higher is better S/N ratio is represented in equation 1. By considering the S/N ratio and ANOVA analysis, the best combination of process parameters are obtained. In order to verify the optimal process parameters the confirmation experiment were done.

$$S/N = -10*\log(\Sigma(1/Y^2)/n) \quad \text{..... (1)}$$

Where y_1, y_2, y_3, ..y_n, are the outcome of the drug release behavior studied, n is the number of observations.

2. Covalent Functionalization.[27,28]

Covalent functionalization was carried to Pristine MWCNTs by the following three methods.

(a) Initial acidic treatment followed by treatment with hydrochloric acid

Covalent functionalized MWCNTs are produced by this method. The initial acidic treatment done by using HNO_3 and H_2SO_4 Which produces oxidized MWCNTs and then same oxidized MWCNTs are treated with HCl and produces carboxylated MWCNTs. 250 mg of MWCNTs were added to a 100 ml mixture of 98% H_2SO_4 and 65% HNO_3 (V:V = 3:1) and agitated for 12 hours at room temperature. After treatment with acids the MWCNTs were completely washed with fresh water and immerse in HCl and refluxed for 24 hours, then acid treated MWCNTs were collected through the filtration

and washed with fresh water to neutral pH. The collected products are dried overnight at 40 ^{O}C in vacuum.

(b) Treatment with conc. Hydrochloric acid

Treatment with conc. Hydrochloric acid is used to purify the CNTs, this method simply purified the CNTs. Take 250 mg of MWNCTs was placed in a 500 ml round bottom flask and 100 ml of HCl was added. The mixture was stirred using magnetic stirrer for 2 hours, then diluted in water, filtered, washed with fresh water and then dried in vacuum at 40 ^{O}C overnight.

(c) Initial basic treatment followed by treatment with hydrochloric acid

Initial basic treatment followed by treatment with hydrochloric acid method is used to produce covalent functionalized MWCNTs. The initial basic treatment done with ammonium hydroxide and hydrogen peroxide to produce oxidized MWCNTs and then the oxidized MWCNTs are treated with HCl to produce carboxylated MWCNTs. 500mg of MWCNTs was mixed with 25 ml of the mixture of ammonium hydroxide (25 %) and hydrogen peroxide (30%) (V: V=1:1) in a 100 ml round bottom flask connected with a condenser and the MWCNTs dispersion was subjected to heating at 800 ℃ and keep aside for 5 hours. Then disperse in HCl and reflux for 12 hours, the treated MWCNTs solution was diluted in water and then filtered. Then the residue was washed with fresh water up to neutral pH and the sample was dried in vacuum at 40 ^{O}C overnight.

Preparation of MWCNTs-Irinotecan complex.[29- 31]

Covalent functionalized MWCNTs were dispersed in Irinotecan solution (Irinotecan in water {25 mg / ml}) at varied concentrations as given in table 1 and sonicated for 30 minutes. Subsequently, the dispersion is 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 deionized water three times and centrifuged to remove free/unbound drug. The supernatant was collected, whereas the solid sample was dried at 30 ^{O}C in a vacuum oven for 24 hours to obtain Covalent Functionalized -MWCNTs- 5-Irinotecan complex. The covalent Functionalized -MWCNTs- Irinotecan complex was stored at room temperature in a vacuum desiccator for further use of studies.

Table 1: DOE for formulations (formulation of covalent functionalized MWCNTs loaded with Irinotecan).

Serial No	Three methods of covalent Functionalized CNTs	Covalent Functionalized CNTs: Drug Conc (Irinotecan) ratio	Covalent Functionalized CNTs: Drug(mg)	Formulations
1	Method 1	1:1	(50:50)	F1
2	Method 1	1:2	(50:100)	F2
3	Method 1	1:3	(50:150)	F3
4	Method 2	2:1	(50:50)	F4
5	Method 2	2:2	(50:100)	F5
6	Method 2	2:3	(50:150)	F6
7	Method 3	3:1	(50:50)	F7
8	Method 3	3:2	(50:100)	F8
9	Method 3	3:3	(50:150)	F9

3. EVALUATION

SEM studies

SEM studies (Model ESEM QUANTA 200) were done at Advanced facility for microscopy and microanalysis, Indian institute of science, Bengaluru by using of Model ESEM QUANTA 200. The scanning electron microscopic techniques are established on the irradiation of the sample by an electron source. Sample was taken, sonicate to break up agglomerates, then use a micro-pipette to deposit a small droplet on a SEM grid and let the solvent evaporate as rapidly as much as possible, sample should be in dry condition for SEM analysis. Observation of sample done 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 steadfastness of this method is regularly limited to objects larger than 1 nm.

TEM Studies

TEM studies were done at Centre for Nano Science and Engineering Indian institute of science, Bengaluru with the help of Model-M3000, Series -3V2413 and Instrument of Titan Themis.The CNT- Drug complexes TEM was used for the study of structural characteristics. Preparation of samples was by using ethanol, 3 to 5 dropsof MWCNs nanosuspension were placed on to a copper grid and the excess liquid was removed by touching one edge of grid with filter paper, then the copper grid with complexes was dried under infrared light and scanned by TEM.

FT-IR Studies

Fourier-transformspectroscopy is a technique used to obtain an infrared spectrum of absorption or emission of a solid, liquid or gas. Clean the sample holder by acetone with Kim wipes, make sure not to splash the acetone on the instrument, take 5 mg of sample and 100 mg of potassium bromide make pellet , keep in pellet holder , scan range is adjusted in the range limit is 400-4000

cm^{-1}, monitor the total pressure applied to the sample, check IR spectra .

Particle size

Particle size was done by using Malvern zetasizer ZS at Malvern-Aimil application centre, Bengaluru. Dynamic light scattering (DLS) method was used for Particle size determination. Dynamic light scattering has become the technique of choice for illustrating nanomaterials due to its speed and ability to readily describe a statistically noteworthy number of particles. The dispersion of sample was vigorously shaken to break up loose aggregate. As such sample (1 ml) is measured with 4sided transparent disposable sizing cuvette, was measured by right angle scattering. The effects of dust (large particle impurities) was suppressed by noise cut features of the software data was analyzed by the technique of cumulants.

Zeta potential

Zetapotential was done by using Malvern zetasizer ZS at Malvern-Aimil applicationcentre, Bengaluru. Colloidal dispersionwere studied with the help of the zeta potential as significant pointer. The magnitude of the zeta potential designates the degree of electro static repulsion between close to like wise charged particles in dispersion. The surface of the CNTs were altered by functionalization, where negative and positive charge lead to electrostatic repulsion between the molecules and stabilize the nanotube colloids. The CNT 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.

TGA procedure

The purity of samples was evaluated via thermogravimetric analysis (TGA) by using Mettler Toledo analyser at Indian institute of sciences, Bangaluru. Thermal property analyses are measured in defining the functional groups in recent times, and TGA is the technique that used widely for this purpose as it is simple and the information can be presented by a simple thermoram. In STAR e software (Mettler Toledo) assign the sample name, heating rate, temperature, purge gas etc. Take empty weight of crucible (Aluminium oxide crucible, 70 microlitre) and then load 1-20 mg 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 finishing the experiment, we have to analyse the curve and save it in folder.

Drug entrapment efficiency

To evaluate a drug entrapment on CNTs, drug entrapment efficiency has to be done to calculate the drug loading. 5 mg of covalent functionalized - MWCNTs- Irinotecan complex was mixed with 10 ml of phosphate buffer pH 7.4, heated at 37 ℃ and then centrifuged at 10,000 rpm for 1 hour 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.

Drug entrapment efficiency (or) drug-loading capacity were calculated according to the following equation

$$\text{Drug} - \text{loadingefficiency}(\%) = \frac{(\text{weight of loaded drug})}{(\text{weight of feeding drug})} * 100 \quad \text{.....(2)}$$

Plan of experiments for drug release

In our study experimental parameters are chosen are a) Drug release time b) covalent functionalized CNT c) Drug conc. d) Buffers . The process parameters and the levels are tabulated in table 2 as per the Taguchi L18 array. 18 tests and each and everyone parameters are varied for 6,3,3 and 3 levels respectively. The drug release data obtained from all formulations according to table no 3. The dialysis memebrane 70 (HIMEDIA) and the freshly prepared dissolution medium Phosphate buffers of pH 6.3,7.4,8.0 were used, take dialysis tube 1.2 inch in length. Take 5 mg drug soak overnight in dissolution medium and fill in pouches formed by the dialysis tube place in the conical flask containing 100 ml of phosphate buffer placed on shaking water bath maintained at 37 ^{O}C with a frequency of 50 shaking per minute. Aliquots, each 1ml volume, were withdrawn according to table 3 with respect to time, aliquots diluted suitably and examined at 254 nm by UV-Vis spectrophotometer.

Table 2: Process parameters and levels.

Level	Drug release time (hr)	Covalent Functionalized CNT	Drug Conc(ratio)	Buffers (PH)
1	2	Method 1	1	6.3
2	8	Method 2	2	7.4
3	18	Method 3	3	8.0
4	28	---	---	---
5	38	---	---	---
6	48	---	---	---

RESULTS AND DISCUSSION

Scanning electron microscope (SEM)

SEM images of covalent FunctionalizedMWCNTs and covalent Functionalized –MWCNTs- Irinotecan complex were obtainable in Figure 1a and 1b respectively. SEM image of Covalentely Functionalized –MWCNTs-Irinotecan complex structures are absolutely different from the covalent Functionalized MWCNTs, the surface and diameter of covalent Functionalized –MWCNTs-Irinotecan complex was increased.

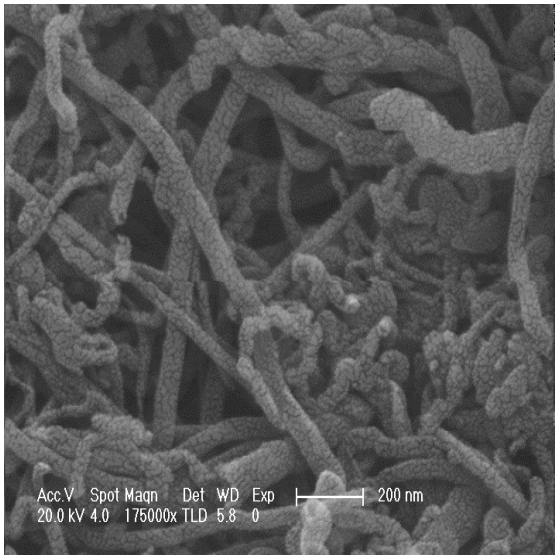


Figure 1a: SEM images of F- MWCNTs.

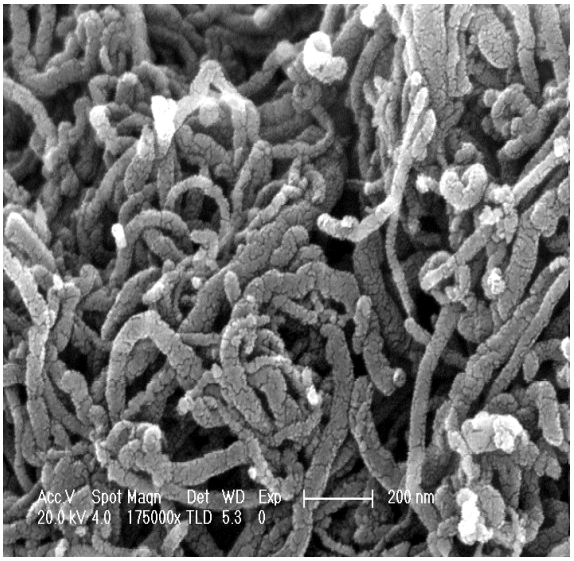


Figure 1b: SEM images of F- MWCNTs-Irinotecan complex.

Transmission electron microscopy (TEM)

TEM images of the covalent Functionalized MWCNTs and covalent Functionalized-MWCNTs- Irinotecan complex are depicted in Figure 2a, and 2b respectively. TEM images exemplify that the tubes are filled with the drug. The outer surface of the tube walls was free from the contaminants. The larger diameter of MWCNTs displays the Irinotecan filler in the tubes.

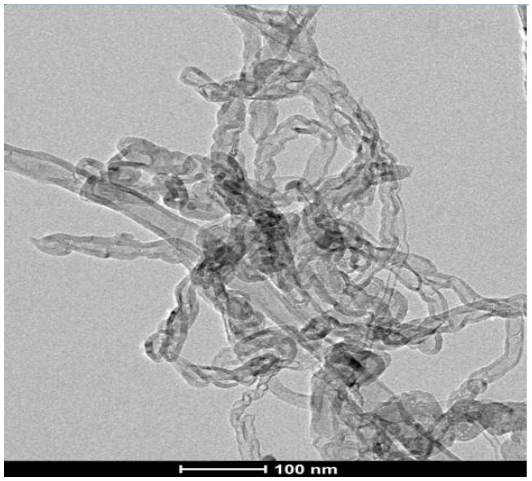


Figure2a: TEM images of F- MWCNTs.

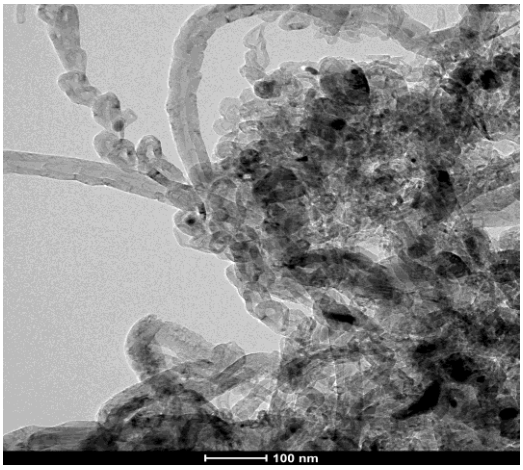
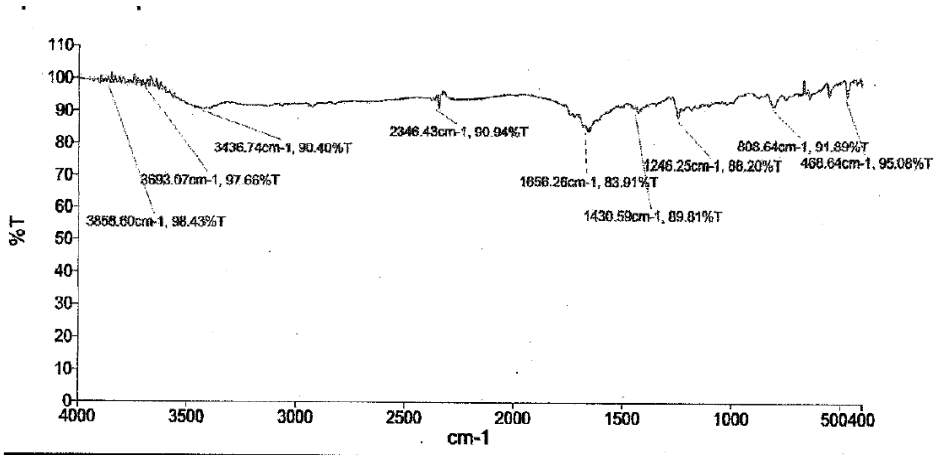


Figure 2b: TEM images of Covalent Functionalized MWCNTs- Irinotecan complex.

Fourier Transform Infrared Spectroscopy (FT-IR)

The sample recorded in the range of 4000 to 400 cm-1. FT-IR spectra of Covalent Functionalized -MWCNTs-Irinotecan complexwas given in the Figure 3. Thecharacteristic peaks showed at 1658.26 cm-1,1430.59 cm-1, 1246.25 cm-1, 808.64 cm-1 due to the presence of C=O, C-H, C-O, C-Cl, functional groups.The peaks for carboxy group at 1710.01 cm-1(range 1740-1700 cm-1) and hydroxyl group at 3130.15 cm-1 (range 3300- 2500 cm-1) approves the pristine MWCNTs go through the covalent functionalization. The sequel provide significant proof of functionalization and linkage of Irinotecan drug to functionalized MWCNTs.



Particle size and Zeta potential

Figure 4a demonstrate the particle size distribution by intensity. The particle size of Covalent Functionalized -MWCNTs- Irinotecan complex is 368.8 (d.nm). Figure 4b presents the zeta potential distribution of Covalent Functionalized -MWCNTs- Irinotecan complex, The zeta potential of Covalent Functionalized -MWCNTs-Irinotecancomplex is -1.66 mV.

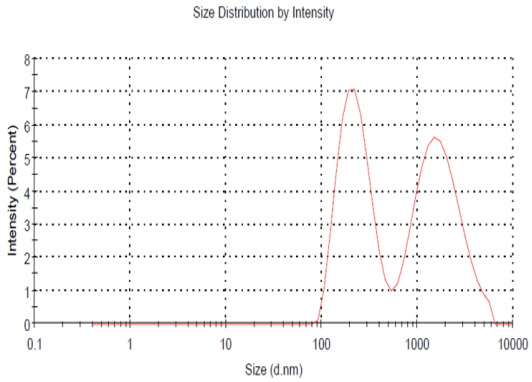


Figure 4a: Particle size Of Covalent Functionalized MWCNTs Irinotecan complex.

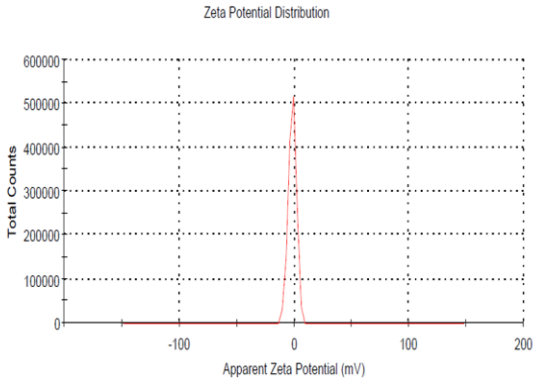
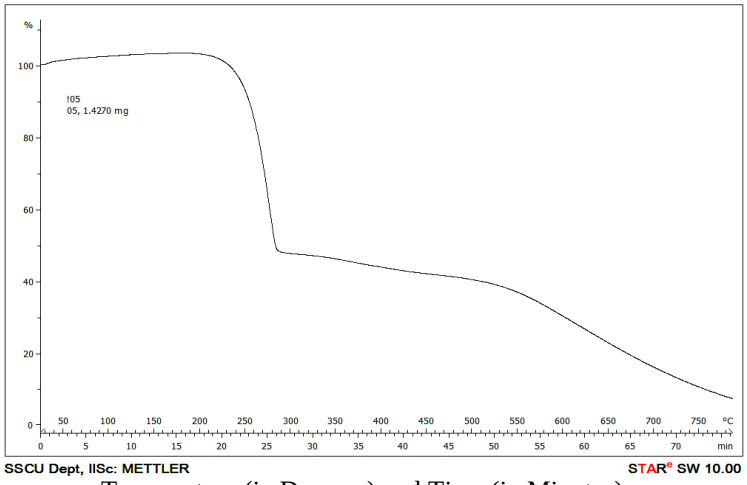


Figure 4b: Zeta potential of Covalent Functionalized MWCNTs- Irinotecan complex.

Thermogravimetric Analysis (TGA)

The Figure 5 depicted the thermo gravimetric (TG) curve signified by the solid lines obtained by the thermo gravimetric analysis of Covalent Functionalized -MWCNTs- Irinotecan complex. Initial burning occurs in the MWCNTs mixture at 220 ^{O}C due to existence of amorphous carbon. The CNTs mixture starts burning themselves and at the end of analysis at 750 ^{O}C the residue remains, mainly due to occurrence of drug found in the CNTs.



Temperature (in Degrees) and Time (in Minutes)

Figure 5: TGA analysis of covalent MWCNTs loaded with Irinotecan.

Drug Entrapment Efficiency

Drug entrapment efficiency was done by Uv spectrum for all the nine formulations of F1, F2, F3, F4, F5, F6, F7, F8 and F9. Uv spectra portrays in figure 6 and shows sensibly good percentage of entrapment efficiency with 60.83%, 65.73%, 66.56%, 67.29%, 79.48%, 83.96%, 88.02%, 84.27%, and 85.00 % respectively.

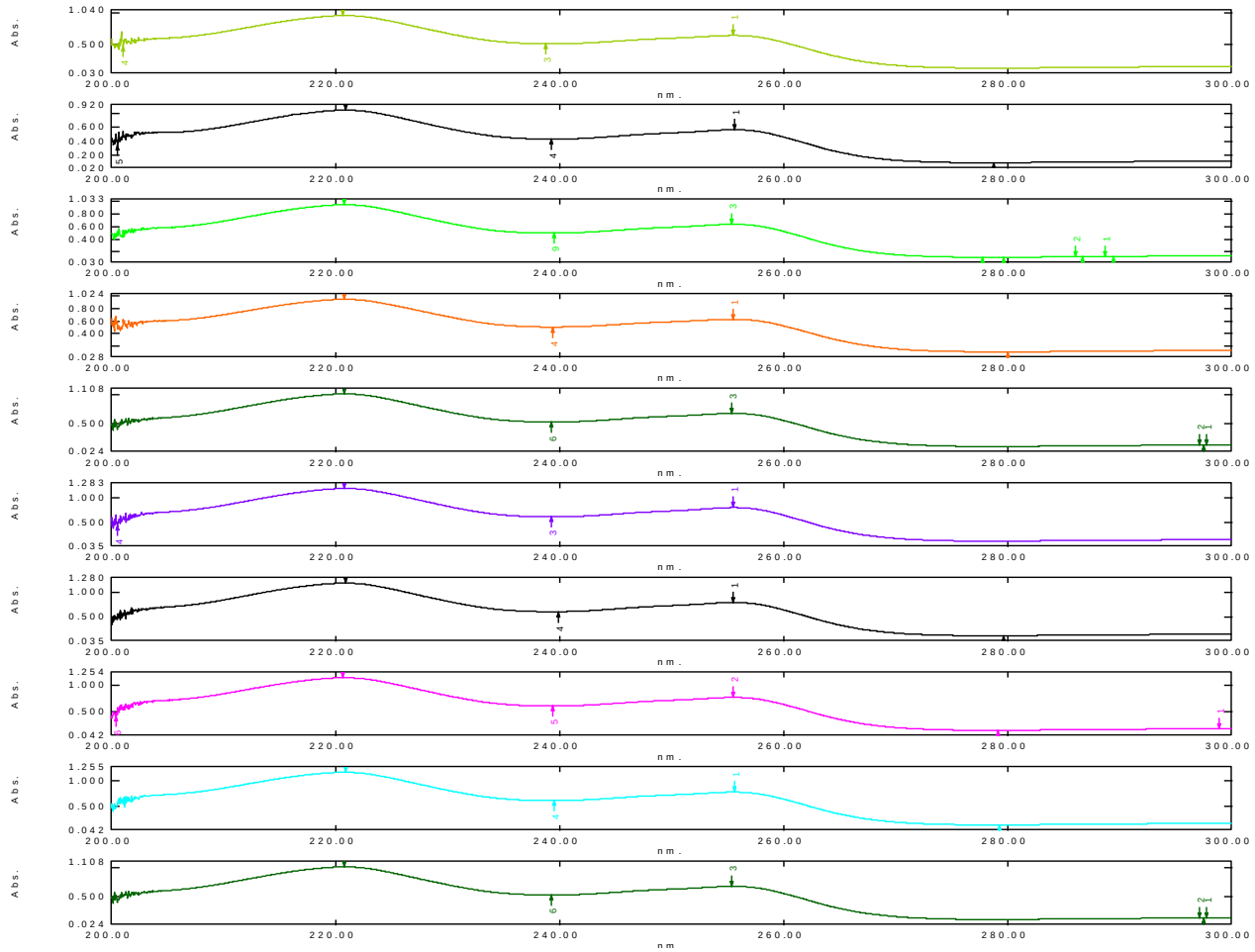


Figure 6: Stacked UV graphs of all 9 formulations of Irinotecan entrapment in F- MWCNTs.

Statistical analysis of drug release studies

The proper OA are preferred for the designing the experiments, based on total number of (DOF) degrees of freedom. Our study consists of 4 factors with 3 levels, total number of degrees of freedom (DOF) = (No of levels -1) × No of main factors = (6 - 1)*1 + (3 - 1) x 3 = 11 ignoring interaction factors. As a result the least number of experimentation = total number of DOF + 1. OA appropriate for our experiments is the which has number of experiments equivalent to or superior than total number of DOF. Therefore the reachable orthogonal array for the DOF condition is L18. The drug release trials were performed as per L18 orthogonal array. The measured results were analyzed using software MINITAB 17. *In-vitro* drug release rates were carried by dialysis method.Table 3 depicts the results of drug release, and validate signal to noise ratio for each one experiment, in experiment no 16 results in best. It is obviously recognized as F3 formulation is the best among the nine batches when likened to other formulations.

By using the Signal to noise ratio the influence of process parameters such as drug release time, functionalized CNTs, drug concentration and buffers has been analyzed and for this process the response table drawn for different combination of parameters was shown in table 6. The rank values afford the input factor influence on response variable. This table enlightens evidently that the time is a principal parameter on drug release then pursed by covalent functionalized CNT, different PH Buffers and Drug concentration, and the same is signified in ANOVA analysis optimized in table 4. In ANOVA table P- Value clarifies which factors are momentous on drug release. P- Value is less than 0.05 for Time so it is noteworthy factor. The R^2- value measures the model degree of fitness, and explains the recital characters are pretentious to great extent by profound factors. The model swift given in table 5 shows the R^2-Value is 96.25%, this gives the authorization that, our model elucidates the connection between input factors and the response variables.

Figure 7 give a picture main effect plot for S/N ratio, this illuminate that as all the factors increases the S/N ratio will also upsurges, maximum S/N ratio is enviable, but as indicated higher the better option. It is obligatory to check the ANOVA while writing the conclusion, the figure 8 illustrates the plot of residuals versus order values does not shows any pattern and therefore constant variance postulation is gratified. Normal Probability plot explains that experimental data get together the normality supposition. In standardized residual versus fits plot, the randomly scattered residuals shows zero. There is no proof of non constant variance, missing terms or outliers exist. It specifies that experimental data satisfies the independent/ randomization assumption. Normal Likelihood plot confirm that the line dividing the points into two equal half hence experimental data get together the normality assumption. From the standardized residual versus the fits plot, the residuals appear to be randomly scattered about zero. No evidence of non-constant variance, missing terms, or outliers exists. Since no unusual structure is apparent, it designates that experimental data about please the independent/randomization assumption is gratified.

Table 03: Result of orthogonal array of Taguchi for Drug Release.

SL. No.	Drug Release Time (hr)	Functionalized CNT (Methods)	Drug Conc (ratio)	Buffers (Ph)	Drug Release (%)	SNRA1
1	2	1	1	6.3	3	9.5424
2	2	2	2	7.4	6	15.5630
3	2	3	3	8.0	6	15.5630
4	8	1	1	7.4	10	20.0000
5	8	2	2	8.0	10	20.0000
6	8	3	3	6.3	12	21.5836
7	18	1	2	6.3	16	24.0824
8	18	2	3	7.4	23	27.2346
9	18	3	1	8.0	28	28.9432
10	28	1	3	8.0	32	30.1030
11	28	2	1	6.3	40	32.0412
12	28	3	2	7.4	40	32.0412
13	38	1	2	8.0	52	34.3201
14	38	2	3	6.3	58	35.2686
15	38	3	1	7.4	59	35.4170
16	48	1	3	7.4	80	38.0618
17	48	2	1	8.0	70	36.9020
18	48	3	2	6.3	61	35.7066

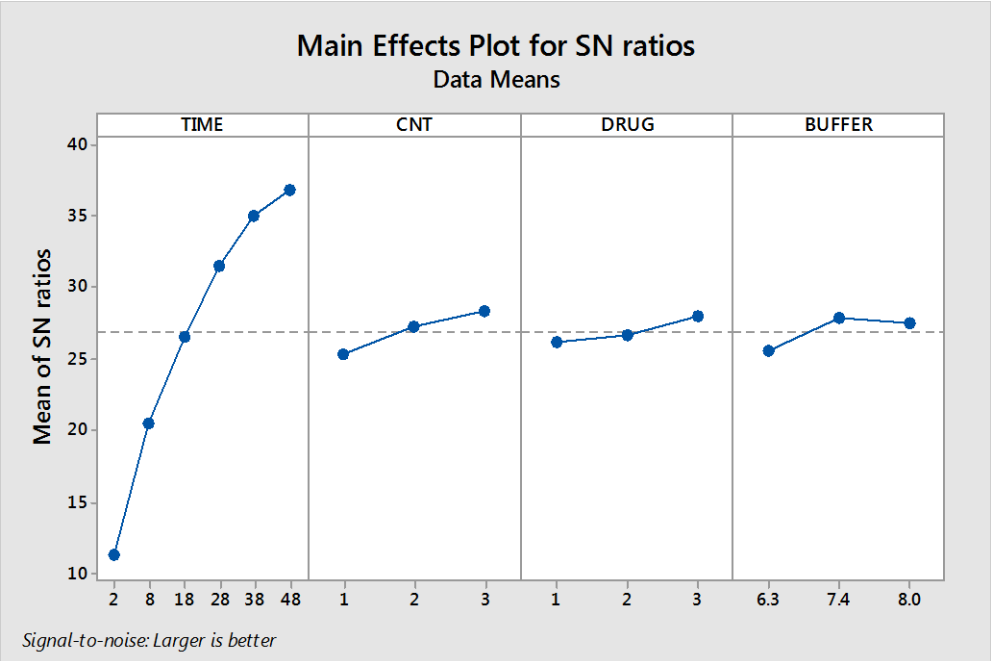


Figure 7: Main effects plot for means.

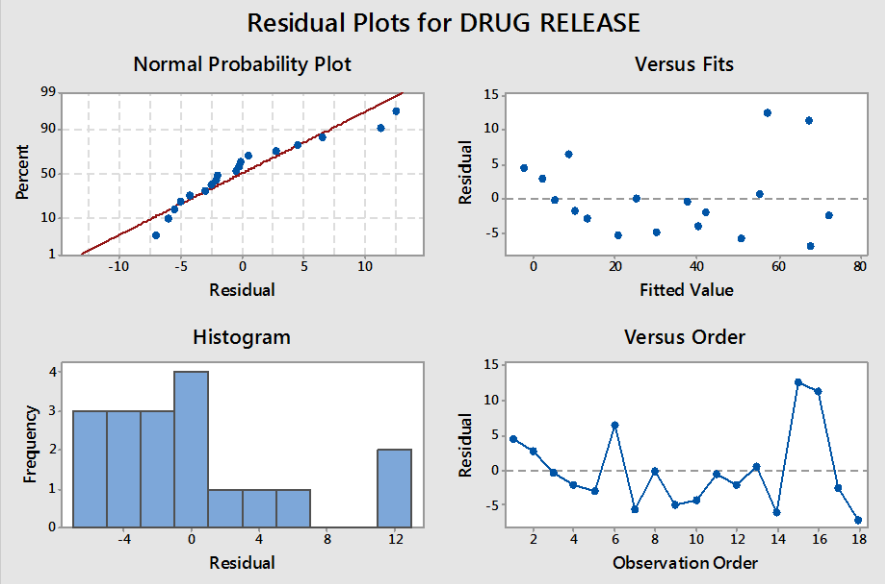


Figure 8: Residual plots for drug release.

Table 4: Analysis of variance (ANOVA).

SL.NO.	Source	DF	Adj SS	Adj MS	F-value	P-value
1	Drug release time	1	9963.9	9936.94	328.08	0.000
2	Functionalized CNT	1	14.1	14.08	0.46	0.508
3	Drug ratio	1	0.1	0.08	0.00	0.959
4	Buffers	1	13.1	13.08	0.43	0.523
5	Error	13	394.8	30.37	---	---
6	Total	17	10386.0	---	---	---

Table 5: Model summary.

R^2-R	Sq(adj)
0.962	0.950

Table 6: Response Table for Signal to Noise Ratios.

Level	S/N ratio of Time	S/N ratio of CNT	S/N ratio of Drug	S/N ratio of Buffer
1	13.56	26.02	27.14	26.37
2	20.53	27.83	26.95	28.05
3	26.75	28.21	27.97	27.64
4	31.40	---	---	---
5	35.00	---	---	---
6	36.89	---	---	---
Delta	23.33	2.19	1.02	1.68
Rank	1	2	4	3

CONCLUSION

Covalent functionalized MWCNTs-Irinotecan complex composed of functionalized MWCNTs loaded with Irinotecan, Carboxylated MWCNTs can be gained lucratively by treating pristine MWCNTs with acids, covalent functionalization boosts the dispersibility of MWCNTs in water. This is new, easy method of distinction can resourcefully stack the drug, lessen the cost and encourage the CNTs in delivery of plenty of drugs. The formulations are characterized by FT-IR studies that authorize the occurrence of functional groups, and the attachment of COOH group. Thermal property examination approves drug loaded to MWCNTs, the residue left over in between 220 ˚C and 750 ˚C, no weight loss below 100˚C that illustrates the residual solvents are absent. MWCNTs after functionalization and drug loading, Particle size increased up to 368.8 (d.nm). Zeta potential was -1.66 mV, negative charge accompany the electrostatic interaction and stabilize the nanotube solution. Drug loaded on MWCNTs proved by microscopic studies and appraised by the drug entrapment efficiency. The drug release obtained from the nanotubes noticed that drug release by the different levels of pH in release medium was in controlled manner and data was statistically

inspected. In summary, for the tumor treatment, these effort of developing MWCNTs- Irinotecan as a drug delivery agent in nanomedicine and enhance the potency of Irinotecan.

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