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PREPARATION, OPTIMIZATION, *IN-VITRO* AND *IN-VIVO* EVALUATION OF FUNCTIONALIZED CARBON NANOTUBES PREDNISOLONE CONJUGATES

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ABSTRACT

This research focuses on developing a novel nanocarrier-based drug delivery system for epilepsy using polyethylene glycol (PEG)-functionalized multi-walled carbon nanotubes (MWCNTs) loaded with Prednisolone. The primary goal was to create a PEG–MWCNT–Prednisolone conjugate that enables sustained and controlled drug release, thereby enhancing therapeutic effectiveness and patient compliance. The MWCNTs were functionalized through a non-covalent method to improve their dispersion stability and drug-loading efficiency. Formulation optimization was conducted using a Design of Experiments (DOE) approach based on the Taguchi method to identify key parameters influencing performance. The conjugates were characterized using Fourier Transform Infrared Spectroscopy (FT-IR) and Scanning Electron Microscopy (SEM). FT-IR confirmed successful attachment of Prednisolone to the functionalized MWCNTs, while SEM images revealed distinct morphological changes on the nanotube surface after drug loading. Particle size analysis showed values ranging from 85 to 650 nm, and a zeta potential of +24 mV indicated good stability of the formulation. The drug entrapment efficiency exceeded 70%, and the cumulative in vitro drug release reached approximately 89%, suggesting an effective sustained-release profile. The experimental design employed two factors at three levels, arranged in an L27 orthogonal array to ensure comprehensive evaluation of formulation variables. The anti-allergy activity produced by optimized formulation show significant increased intact cells in mast cell stabilizing. Statistical analysis validated the consistency and reliability of the experimental data. Overall, the PEG-functionalized MWCNT–Prednisolone conjugate showed excellent dispersion, stability, and prolonged release characteristics, indicating its strong potential as an advanced drug delivery system for allergy therapy. This study highlights the promising role of functionalized carbon nanotubes in improving the pharmacological performance of conventional anti-allergy drugs.

KEYWORDS: Polyethylene glycol, Multi Walled Carbon Nanotubes, Design of Experiments, Scanning Electron Microscopy, Mast cell stabilizing, allergy

INTRODUCTION

Carbon nanotubes, first discovered by Iijima in 1991, are made up of thin sheets of benzene ring carbons rolled up into the shape of a seamless tubular structure. The CNT structure belongs to the family of fullerenes, the third allotropic form of carbon along with graphite and diamond[1]. CNTs are allotropes of carbon. They are tubular in shape, made of graphite. They are nanometers in diameter and several millimeters in length and have a very broad range of electronic, thermal, and structural properties[2]. Carbon nanotubes (CNTs) are cylindrical nanostructures composed of carbon, typically with diameters measured in nanometers. They are essentially formed from graphite sheets, where continuous, unbroken hexagonal networks of carbon atoms appear as rolled-up layers. They are long, hollow structure with walls made of graphene, a single atom–thick sheet of carbon[3].

CNTs are similar to graphite and are composed of sp^2 carbon bonds, which are stronger than the sp^3 bonds found in diamonds. The sp^2 bonding defines the unique energy properties of CNTs, and through van der Waals interactions, individual nanotubes assemble into bundles or ropes[4]. A carbon nanotube may consist of one or more cylindrical layers of graphitic sheets also known as graphene. They are bonded to three neighboring carbon atoms through sp2 hybridization to form a continuous and seamless tubular structure. The outer diameter varies typically between 2-20 nm in the arc-produced nanotubes, while the inner hollow diameter is typically on the order of 1-3 nm [5].

CNT's can be functionalized to attain desired properties that can be used in a wide variety of applications. Because of their hydrophobic nature, CNT's tend to agglomerate hindering their dispersion in solvents. The resulting nanotube bundles or aggregates reduce the mechanical performance of the final composite. The surface of CNT's can be modified to reduce the hydrophobicity and improve interfacial adhesion to a bulk polymer through chemical attachment [6]. CNTs have significant applications, particularly in the miniaturization of medical devices, the advancement of electronic systems, and the development of nanotools designed to interact with the human body and monitor complex biological processes[7]. Nanotubes are considered as nearly one-dimensional structures according to their high length to diameter ratio. Most important structures are single walled nanotubes (SWNTs) and multi walled nanotubes (MWNTs). Carbon

nanotubes have fullerene like structure and having graphene sheets which contain sp2 hybridisation of each carbon atom [8]. CNTs are part of the fullerene family, which are a group of carbon allotropes with atoms linked in the shape of cage-like structures such as a hollow sphere, ellipsoid or cylindrical tube. Fullerenes are comprised of graphene sheets linked with hexagonal and pentagonal rings, which give their curved structure [9].

Carbon nanotubes are also called "rulers of nanomaterials" which are the carbon allotropes with diverse carbon-carbon bonds, seamless tubes of graphene sheets which are resulting from a honeycomb lattice with the surface length to width ratio will be more than 1,000,000 and a hundred times stronger than steel with distinct physical and chemical properties with nanosized structures[10]. Carbon Nanotubes (CNTs) have become strongest candidates mainly in the field of biomedical engineering, biotechnology; defense research and pharmaceutical nanotechnology after their discovery in 1991. These are the long hollow seamless cylinders (single walled as well as multiwalled carbon Nanotubes) of graphene. The diameter of these tubes in the range of 1-100 nm. These tubes are normally capped with the half a full fullerene molecules at both the ends[11].

Prednisolone has a relatively short plasma half-life (2–4 hours), which necessitates frequent dosing to maintain therapeutic drug levels. This leads to fluctuations in plasma concentration, reduced patient compliance, and an increased risk of adverse effects due to peak–trough variations. Our aim of this research work is converting prednisolone into a sustained-release formulation by loading it into multiwalled carbon nanotubes (MWCNTs) with the following advantages: The hollow cylindrical structure and high surface area of MWCNTs allow for efficient drug loading and gradual release.This is especially useful for corticosteroids like prednisolone, which undergo extensive hepatic metabolism. Avoiding sharp plasma peaks minimizes corticosteroid-related adverse effects such as hyperglycemia, immunosuppression, and gastric irritation. Encapsulation in MWCNTs protects prednisolone from premature degradation in biological fluids, improving therapeutic efficacy. MWCNTs can be co-loaded with anti-inflammatory agents, antioxidants, or targeting ligands, enabling synergistic therapeutic outcomes.

MATERIALS

The following drug were obtained from commercial suppliers and used as received: Prednisolone (Strides yarrow chem pvt ltd), Carbon nano tubes (Nano wings pvt. Ltd Hyderabad). PEG and all chemicals used laboratory grade purchased vasa scientific, Bangalore.

Methods

Functionalization of MWCNT's [12]

To prepare PEGylated MWCNTs, disperse 1500 mg of pristine multi-walled carbon nanotubes (MWCNTs) in 100 mL of PEG solution at concentrations of 5%, 10%, and 15% separately. Transfer each mixture to a sonicator tube and subject it to ultrasonication using a fast clean probe sonicator for 30 minutes to ensure thorough dispersion. After sonication, centrifuge the suspension at 3000 rpm to separate unbound and agglomerated PEG. Carefully discard the supernatant and collect the pellet containing the PEGylated MWCNTs. Wash the pellet with distilled water to remove residual PEG, then filter the suspension through a 0.2-micron Millipore vacuum filter for further purification. Finally, collect and dry the PEG-functionalized MWCNTs completely for subsequent applications.

Binding of Prednisolone To PEG Functionalized MWCNT's

PEG-functionalized multi-walled carbon nanotubes (MWCNTs) at concentrations of 0.5%, 1%, and 1.5% were combined with prednisolone in a 1:1 ratio (w/w) and sonicated for 30 minutes to ensure uniform dispersion. The resulting suspensions were then incubated on an orbital shaker for 1–3 days to promote efficient drug loading onto the functionalized MWCNTs. Following incubation, the dispersions were air-dried at room temperature for 24 hours to yield the PEG-MWCNT–prednisolone conjugates, which were subsequently stored in a vacuum desiccator at room temperature until use in further experiments.

Design Of Experiments [13,14]

Statistical method: Taguchi Method, For designing the experiments an appropriate (orthogonal array) OA needs to be selected, based on total number of degrees of freedom (DOF). Since the present study considers 2 factors with 3 levels, If we consider interaction effects then

Total number of DOF =(No of levels - 1) x No of Main factors + DOF of interaction

= (3 - 1) x 2 + (3-1)*(3-1)

= 8. (Therefore minimum number of experiments = total number of DOF + 1.)

Hence suitable OA is the one which has number of experiments equal to or greater than total number of DOF. Hence L27 OA was selected and physical layout for the experimentation.

Table no.1 Experimental Run of formulations (Design of Experiments)

Experimental run	Drug conc (mg)	Binding time (days)	PEG 6000 F-MWCNT's
F1	5	1	0.5 %
F2	5	1	0.5%
F3	5	1	0.5%
F4	5	2	1 %

F5	5	2	1%
F6	5	2	1%
F7	5	3	1.5 %
F8	5	3	1.5%
F9	5	3	1.5%
F10	5	1	0.5 %
F11	5	1	0.5%
F12	5	1	0.5%
F13	5	2	1 %
F14	5	2	1%
F15	5	2	1%
F16	5	3	1.5 %
F17	5	3	1.5%
F18	5	3	1.5%
F19	5	1	0.5 %
F20	5	1	0.5%
F21	5	1	0.5%
F22	5	2	1 %
F23	5	2	1%
F24	5	2	1%
F25	5	3	1.5 %
F26	5	3	1.5%
F27	5	3	1.5%

Evaluation of PEG Functionalized MWCNTs Prednisolone (PN) Conjugate

Drug entrapment efficiency[15]

To determine the drug entrapment efficiency and loading capacity of the formulation, 5 mg of covalently functionalized MWCNT–Prednisolone conjugate was dispersed in 10 ml of methanol, incubated at 37°C, and centrifuged at 5000 rpm for 1 hour to extract the entrapped drug from the MWCNT matrix. One milliliter of the supernatant was appropriately diluted, and the concentration of Prednisolone was quantified using a UV–visible spectrophotometer at 246 nm. The encapsulation efficiency and drug-loading capacity were calculated using the following equations.

Drug − loading efficiency (%) =weight of loaded drug)/(weight of feeding drug) × 100

In vitro release studies[16]

The in vitro drug release profile of all formulations was evaluated using a dialysis membrane (Dialysis Membrane-70, HiMedia). Phosphate buffer (pH 7.4) was used as the dissolution medium. An accurately weighed quantity of each formulation equivalent to 5 mg of Prednisolone was placed into a dialysis tube (approximately 1.2 inches in length) that had been pre-soaked overnight in the dissolution medium, and the ends were securely tied to form a pouch. The dialysis pouches were immersed in conical flasks containing 100 ml of phosphate buffer (pH 7.4) maintained in a shaking water bath at 37°C with a frequency of 50 shakes per minute. At predetermined time intervals, 2 ml aliquots were withdrawn and replaced with an equal volume of fresh dissolution medium to maintain sink conditions. The collected samples were suitably diluted and analyzed spectrophotometrically at 246 nm to determine Prednisolone release.

% CDR = (Total loaded drug − each time interval released drug + previous total released drug)/(Total loaded drug) ∗ **100**

FT-IR Studies[17]

Fourier-transform infrared (FTIR) spectroscopy was employed to obtain the infrared spectra of the samples in the range of 400–4000 cm^{-1}. Prior to analysis, the sample holder was cleaned using acetone and Kimwipes to avoid contamination, ensuring that acetone did not come into contact with the instrument surface. Approximately 5 mg of the sample was blended with 100 mg of spectroscopic-grade potassium bromide (KBr) and compressed into a transparent pellet using a hydraulic press. The prepared pellet was then placed in the sample holder, and the spectra were recorded under controlled pressure conditions to ensure optimal signal quality.

Particle size[18]

Dynamic light scattering (DLS) was employed to determine the particle size of the nanodispersions. The sample dispersion was vigorously shaken prior to analysis to disrupt loosely bound aggregates. An aliquot of 1 ml was transferred to a clean, four-sided disposable transparent cuvette, and measurements were performed at a right-angle (90°) scattering geometry. Instrument software features were used to minimize the influence of dust and large particulate contaminants, and the autocorrelation data were evaluated using the cumulants method to obtain the hydrodynamic particle size.

Zeta potential[19]

Zeta potential was measured to assess the colloidal stability of the carbon nanotube (CNT) dispersions. Zeta potential reflects the magnitude of electrostatic repulsion between similarly charged particles and is therefore indicative of the degree of dispersion stability. Surface functionalization of CNTs introduced charged groups, generating electrostatic repulsion that helped to stabilize the nanotube colloids. For measurement, the CNT dispersion was sonicated at 90 Hz for 15 minutes, allowed to stand at room temperature for 24 hours, and then 1 ml of the undiluted sample was transferred into a disposable folded capillary cell for zeta potential analysis.

SEM Studies[20]

Scanning electron microscopy (SEM) analysis was performed by irradiating the sample with a focused electron beam under high vacuum conditions (~1×10^{-3} Pa). The sample was sonicated to disperse any aggregates, and a small droplet of the suspension was deposited onto an SEM grid using a micropipette. The solvent was allowed to evaporate rapidly to ensure the sample was dry before imaging. The electron beam was scanned across the specimen surface, and backscattered electrons were detected to generate high-resolution images reflecting the morphology and topography. The resolution of SEM typically enables visualization of features larger than 1 nm.

In vivo release studies[21,22]

Experimental Animals

Wistar albino rats weighing between 150 and 250 g were used in the study. The animals were housed in standard cages at a room temperature of 25 ± 2°C under a 12-hour light-dark cycle. After an acclimatization period, the rats were randomized into experimental and control groups and placed individually in sanitized polypropylene cages lined with sterile paddy husk bedding. They were provided free access to standard pellet feed and water ad libitum. All animal care and experimental procedures were conducted in accordance with the guidelines of the Committee for the Control and Supervision of Experiments on Animals (CPCSEA).

Grouping of animals for Compound 48/80- induced mast cell degranulation on rat mesentry

Groups	Treatment
I	Normal saline (10 ml/kg, p.o.)
II	Compound 48/80 (1 mg/kg, s.c.)
III	Compound 48/80 (1 mg/kg, s.c.) + prednisolone (5mg/kg ip)
IV (n=12)	Compound 48/80 (1 mg/kg, s.c.) + Formulation MWCNT conjugate (5mg/kg, i.p.).

Procedure:

Albino Wistar rats of either sex, weighing between 180 and 200 g, were divided into four groups. The first three groups each contained six animals, while the fourth group comprised twelve animals, with two animals sacrificed at each predetermined time interval. Group I received normal saline (10 ml/kg, p.o.). On day one, Group II rats were treated with Compound 48/80 (1 mg/kg, s.c.), Group III rats were sensitized with Compound 48/80 (1 mg/kg, s.c.) and administered prednisolone (5 mg/kg, i.p.) as the reference standard, and Group IV received Compound 48/80 (1 mg/kg, s.c.) followed by the Carbon Nanotube-Prednisolone conjugate (5 mg/kg, i.p.).On the seventh day, rats were sacrificed at different time intervals (2, 4, 6, 8, and 12 hours) post-treatment, and the intestinal mesentery was collected for mast cell analysis. The mesenteries, along with intestinal tissue pieces, were spread on Petri dishes containing Ringer Locke's solution at 37°C, transferred to slides, and stretched using needles. Intestinal tissue pieces were removed. Mesentery samples were then challenged in vitro with 5 µg/ml of Compound 48/80 solution for 10 minutes, followed by staining with 0.1% toluidine blue in 4% aqueous formalin. The stained samples were immersed in xylene for 5–10 minutes, rinsed two to three times with acetone, and examined under a microscope at 45× magnification. A total of 100 mast cells per sample were counted from different fields, with intact and degranulated cells quantified. Percentage protection was calculated using the formula:

$$\%\text{Protection}=[1-T/C]\times 100$$

Where,

T represents the number of degranulated cells in the test group, and

C denotes the number of degranulated cells in the inducer control group.

RESULTS

Table. 3 Drug Entrapment Studies of F1 to F27 formulations

Formulation Code	% Entrapment	Formulation Code	% Entrapment	Formulation Code	% Entrapment
F1	68%	F10	72%	F19	72%
F2	66%	F11	70%	F20	75%
F3	69%	F12	71%	F21	68%
F4	68%	F13	74%	F22	72%
F5	70%	F14	77%	F23	75%
F6	69%	F15	74%	F24	76%
F7	72%	F16	73%	F25	78%
F8	74%	F17	77%	F26	81%

F9	71%	F18	79%	F27	83%

Table. 4 In Vitro Drug Release Studies of F1 to F27 formulations

Formulation Code	% CDR	Formulation Code	% CDR	Formulation Code	% CDR
F1	83±0.75	F10	85.3±0.37	F19	69.3±0.7
F2	84±0.65	F11	86±0.59	F20	68.3±0.8
F3	83.3±0.7	F12	88±0.59	F21	70.2±0.4
F4	86±0.46	F13	89±0.59	F22	72±0.63
F5	85..3±0.58	F14	87.9±0.45	F23	74.9±0.31
F6	86±0.55	F15	89.0±0.4	F24	70.5±0.4
F7	88±0.58	F16	85.3±0.59	F25	77±0.82
F8	86.9±0.68	F17	86±0.65	F26	72±0.5
F9	87.6±0.86	F18	88±0.65	F27	71±0.6

Table. 5 Experimental results with computed S/N ratio

Expt. No.	Drug release parameters						
	Rotation Days R	PEG% P	Trial 1	Trial 2	Trial 3	Mean	S/N ratio
1	1	0.5	83	84	83.3	83.4333	38.4265
2	1	1.0	86	85.3	86	85.7667	38.6662
3	1	1.5	88	86.9	87.6	87.5000	38.8398
4	2	0.5	85.3	86	88	86.4333	38.7314
5	2	1.0	89	87.9	89	88.6333	38.9515
6	2	1.5	85.3	86	88	86.4333	38.7314
7	3	0.5	69.3	68.3	70.2	69.2667	36.8088
8	3	1.0	72	74.9	70.5	72.4667	37.1946
9	3	1.5	77	72	71	73.3333	37.2898

Table. 6 Response table for S/N Ratios

Level	rotation day	PEG 6000 %
1	38.64	37.99
2	38.80	38.27
3	37.10	38.29
Delta	1.71	0.30
Rank	1	2

Table. 7 Result of Analysis of variance (ANOVA) for response variable

Source	DF	Adj SS	Adj MS	F-Value	P-Value	% Contribution
Regression	2	186.944	93.472	7.55	0.003	38.62%
Rotation day	1	186.889	186.889	15.10	0.001	38.6%
PEG	1	0.056	0.056	0.00	0.947	1.2%
Error	24	297.130	12.380			
Total	26	484.074				

Table . 8 Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)

4.66367	64.50%	59.86%	52.75%

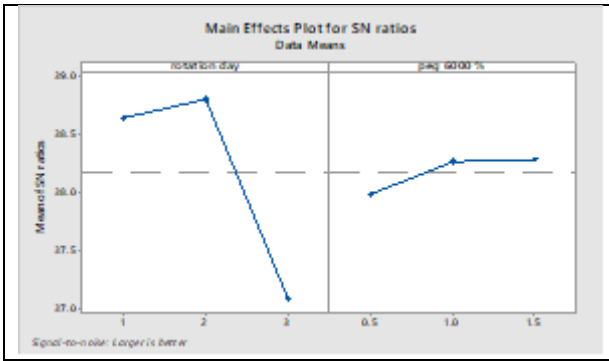


Fig.1a Main Effects plot for S/N Ratios (Data Means)

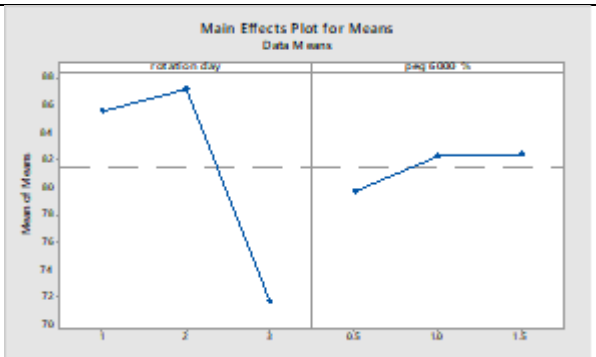


Fig.1b Main effects plots mean

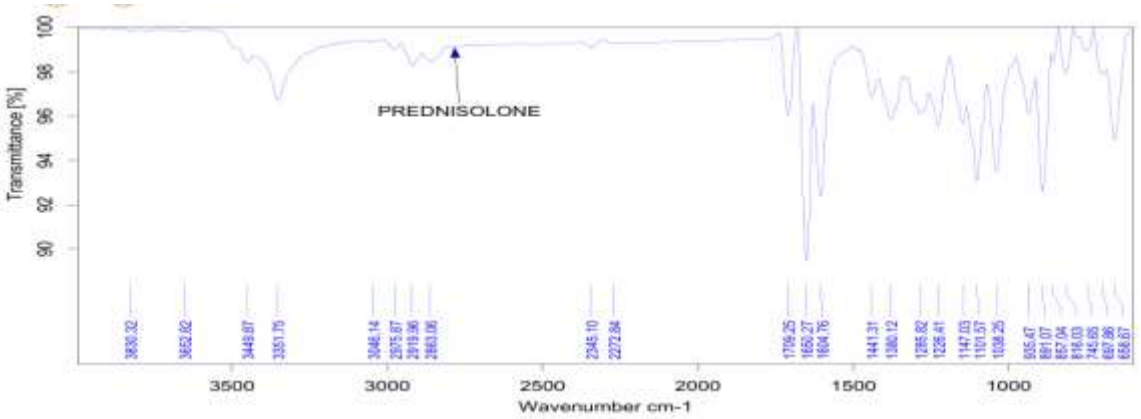


Fig. 2 FT-IR spectrum of optimized formulation F18

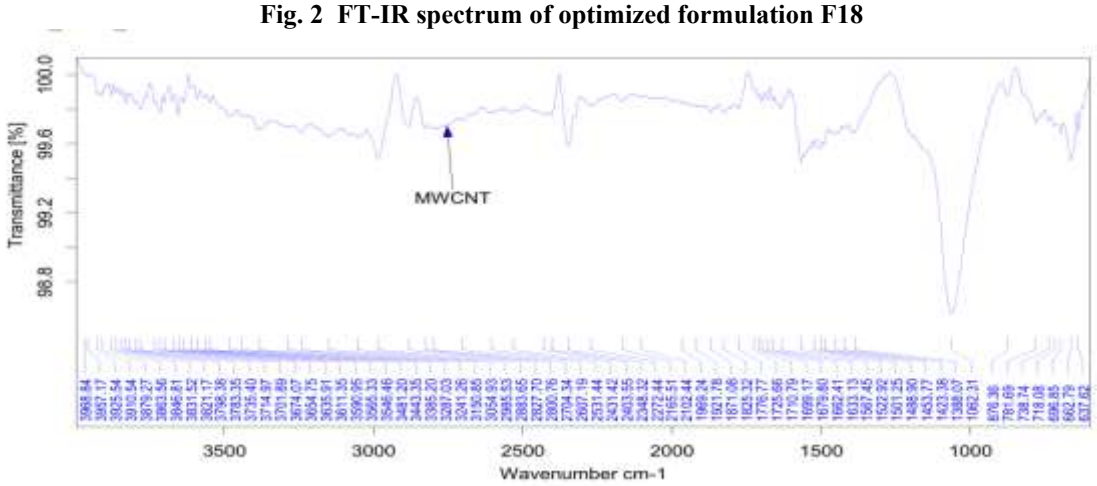


Fig. 3 FT-IR spectrum of pure prednisolone

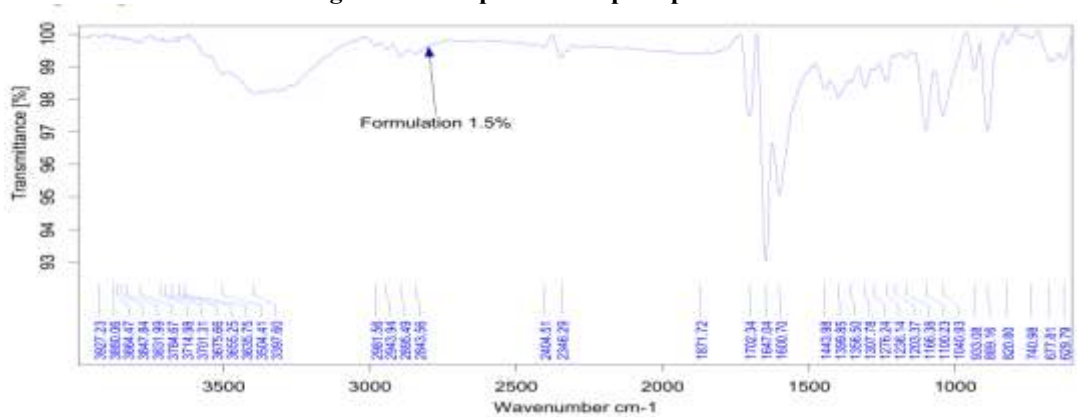


Fig. 4 FT-IR spectrum of MWCNT

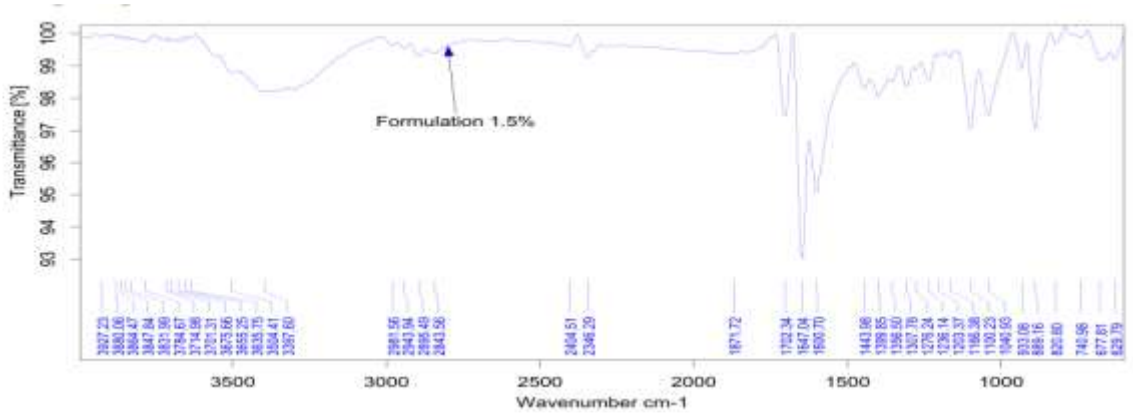


Fig. 5 FT-IR spectrum of optimized formulation F18

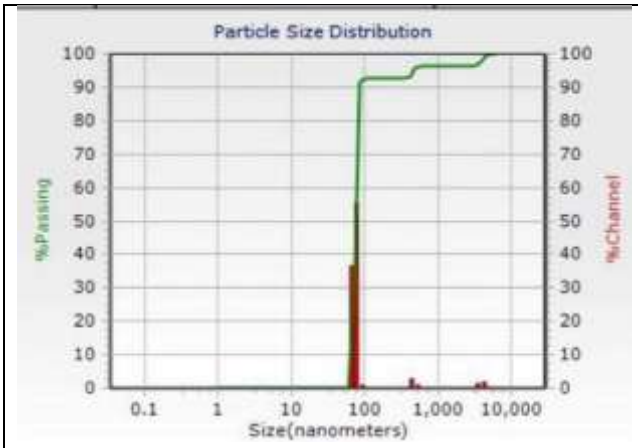


Fig.6 Particle Size Distribution of MWCNTs

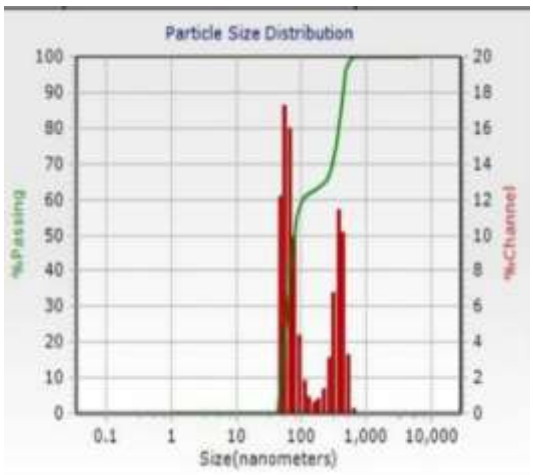


Fig.7 Particle Size Distribution of Optimized Formulation F18

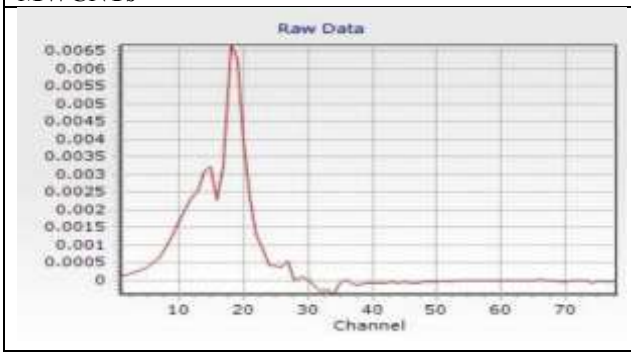


Fig. 8 Zeta potential of MWCNTs

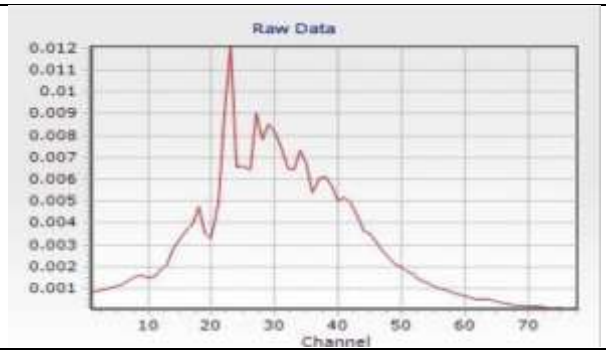


Fig. 9 Zetapotential of Optimized Formulation F18

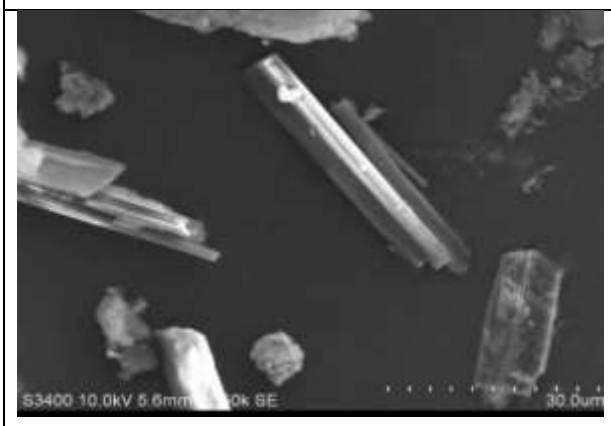


Fig.10 SEM Images of Optimized Formulation F18

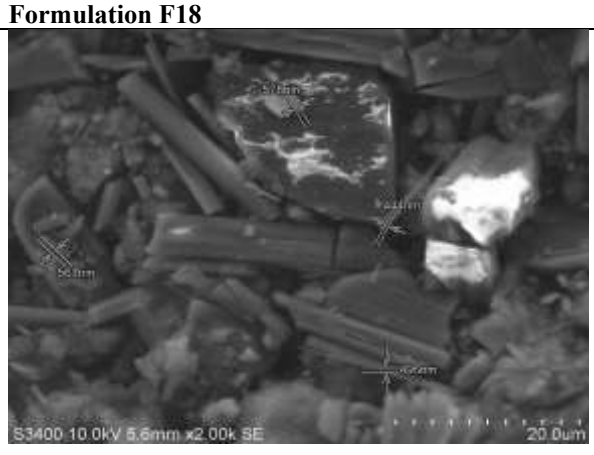
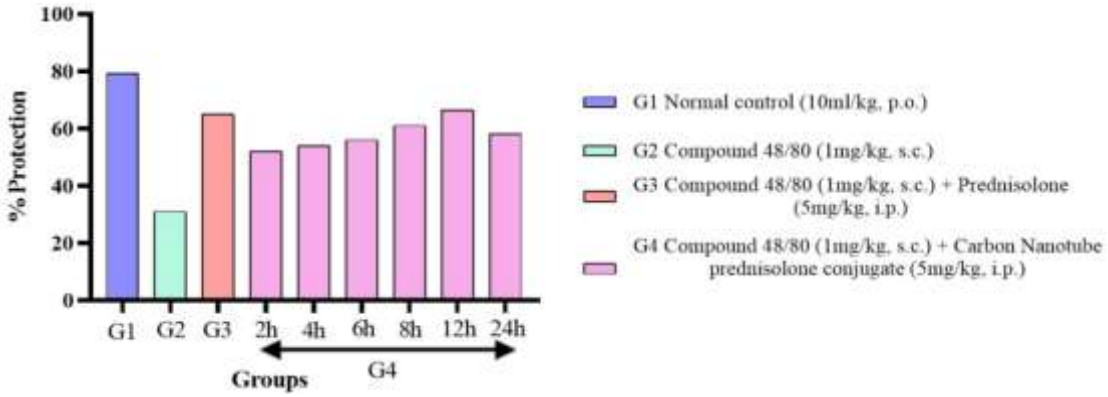


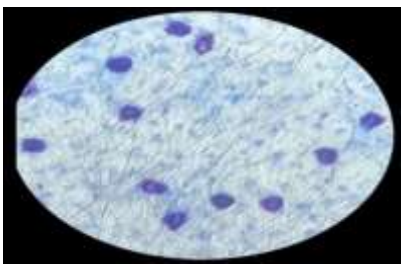
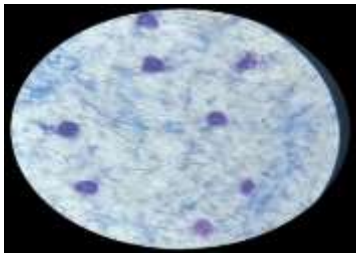
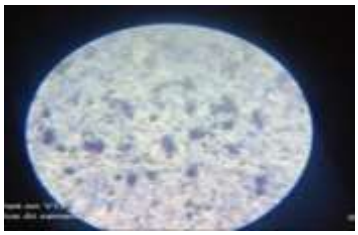
Fig.11 SEM Images of Optimized Formulation F18

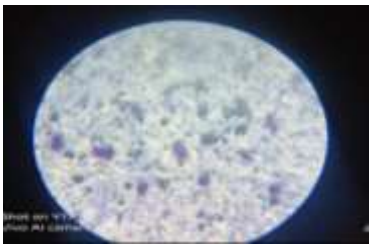
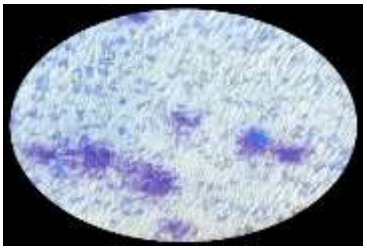
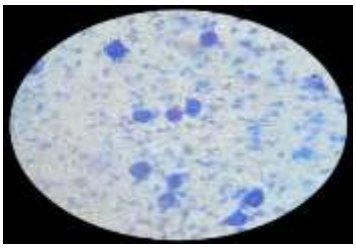
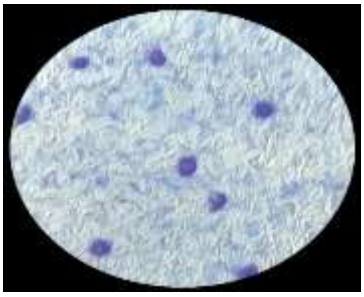
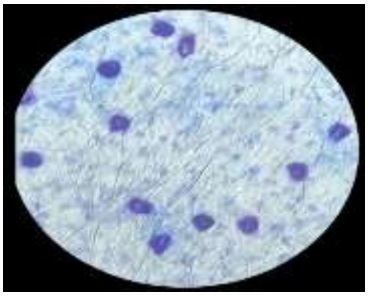
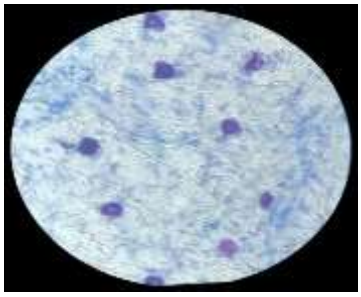
Table 14-Effect of prednisolone on Compound 48/80- induced mast cell

Group	Treatment	% of intact Cells	% of degranulated cells	% Protection
1	Normal Control (Vehicle 10ml/kg p,o)	79.33 ± 0.94	0.67±0.4944	79.33 %
2	Inducer Control (C48/80, 1mg/kg s,c)	331.00±1.438	69.00±1.438	
3	Compound 48/80 (1mg/kg,s.c.) +prednisolone(5mg/kg ip)	65±1.54	34±0.57	65%
4	Compound 448/80 (1 mg/kg, s.c.) + Carbon Nanotube Prednisolone conjugate (5mg/kg, i.p.).	2 Hours		
		52±1.500	47.50±1.500	52.5%
5	Compound 448/80 (1 mg/kg, s.c.) + Carbon Nanotube Prednisolone conjugate (5mg/kg, i.p.).	4 hours		
		54±0.5	45±0.447	54%
6	Compound 48/80 (1 mg/kg, s.c.) + Carbon Nanotube Prednisolone conjugate (5mg/kg, i.p.).	6 hours		
		56±1.500	43.50±1.450	56..5%
7	Compound 48/80 (1 mg/kg, s.c.) + Carbon Nanotube Prednisolone conjugate (5mg/kg, i.p.).	8 hours		
		61±1.500	38.50±1.500	61..5%
8	Compound 48/80 (1mg/kg, s,c) + carbon nanotube prednisolone conjugate (5mg/kg i,p)	12 hours		
		66.50±1.500	33.50±1.490	66..5%
9	Compound 48/80 (1mg/kg, s,c) + Carbon Nanotube Prednisolone conjugate (5mg/kg i.p)	24 hours		
		58±2.000	45.00±1.000	57%



Effect of Prednisolone in C-48/80 induced mast cell degranulation on rat mesentry in vivo

		
G1 – Normal Controol	**G2 - Inducer Control C48/80**	**G3 – Standard Prednisolone**

		
G4 – Formulation 2 hours	**G4 Formulation – 4 hours**	**G4 Formulation – 6 hours**
		
G4 – Formulation 8 Hours	**G4 – Formulation 12 hours**	**G4 – Formulation 24 hours**

DISCUSSION

Multiwalled carbon nanotubes (MWCNTs) are cylindrical nanostructures composed of multiple concentric graphene layers, they have gained considerable attention as advanced nanocarriers and structural materials due to their high aspect ratio, large surface area, and unique hollow core architecture Their ability to encapsulate or adsorb therapeutic molecules, coupled with surface functionalization strategies, allows for improved solubility, biocompatibility, and targeted delivery. Moreover, MWCNTs can be engineered to offer sustained and controlled drug release, which is particularly advantageous for drugs with short half-lives or those requiring long-term administration. Sustained-release formulations of prednisolone decrease dosing frequency, which is especially beneficial for chronic conditions requiring long-term corticosteroid therapy. Functionalization of multiwalled carbon nanotubes (MWCNTs) is a critical step to improve their solubility, biocompatibility, and dispersion stability, particularly for biomedical applications. Among various approaches, polyethylene glycol (PEG) functionalization has been widely adopted due to its hydrophilic nature, low toxicity, and ability to reduce immunogenicity. It Enhances water solubility and colloidal stability of MWCNTs, Reduces opsonization and clearance by the reticuloendothelial system (RES), thereby prolonging circulation time in vivo. To optimize the loading of prednisolone into PEG-functionalized multiwalled carbon nanotubes (MWCNTs), a Design of Experiments (DOE) approach was applied using the Taguchi method in Minitab software. The Taguchi method is a robust statistical tool that reduces the number of experiments required while providing reliable information on factor influence and interaction.

In this study, two critical process factors were selected:

1.Rotation time (days) – representing the duration of mixing/incubation during drug loading.

2.Degree of PEG functionalization of MWCNTs – influencing dispersibility and surface availability for drug adsorption. Each factor was varied at three levels, thereby capturing the lower, medium, and higher settings of both parameters. Although two factors with three levels each can be studied using an L9 orthogonal array, the L27 array was chosen to provide higher resolution and improved error estimation, as well as to accommodate potential interactions between the factors.

The orthogonal array (L27) generated 27 experimental runs with different combinations of rotation days and PEG-functionalization levels. 27 formulations of PEG Functionalized-MWCNTs-conjugates were prepared according to DOE. The results of drug entrapment studies demonstrated that all formulations exhibited good drug entrapment from 68% to 83%. This finding indicates that both rotation duration and degree of PEG functionalization play an important role in improving drug entrapment by enhancing drug–nanotube interaction and dispersion stability.

The FTIR spectra of Prednisolone, MWCNTs, and Prednisolone-loaded PEG functionalized MWCNTs show no significant shift in the major characteristic peaks. The retention of key functional peaks (O–H, C=O, C=C, and C–O) indicates that no chemical interaction or bond formation occurred between the drug and the carrier.The absence of new peaks or major shifts suggests that Prednisolone is physically entrapped or adsorbed onto the PEG-functionalized MWCNTs rather than chemically conjugated.The comparative FTIR analysis confirms that: The functional groups of Prednisolone remain intact after loading. PEG functionalization and drug loading do not alter the fundamental chemical structure of MWCNTs.No shift in the characteristic peaks verifies the compatibility between Prednisolone, PEG, and MWCNTs, indicating successful physical loading of the drug onto the carrier system

The response variable was drug release studies, which was quantified for each run in laboratory. The data were analyzed in Minitab using Signal-to-Noise (S/N) ratios, with the criterion “larger-the-better” since the objective was to maximize drug loading. Main effects plots and analysis of variance (ANOVA) were then used to determine the relative influence of each factor and to select the optimized formulation from 27 formulations. The experimental results were analyzed using ANOVA with the objective of identifying the factors that significantly affects the Drug Release. The sum of squares in ANNOVA Table 4 indicates that Rotation Day has a largest contribution to the total sum of squares [(866.67/1408.95) x 100 = 61.54%] and the contribution of PEG Concentration to the sum of Square is [(42.04/1484.074) x 100 = 2.98%]. which means that it is the major factor influence S/N ratio is Rotation Day followed by the PEG Concentration which makes only a contribution of (2.98%).

On the other hand the larger factor F-value also indicates its larger effect, here Rotation Day has larger f values hence effects the Drug release more. During the analysis significance level of a = 0.05, i.e., for a confidence level of 97% was considered. ANNOVA table also shows factors with a P-value less than 0.05 (Rotation Day) which statistically has a significant contribution towards Drug Release.Regression model was constructed using MINITAB 19 with Drug Release a function affected by process parameters like Rotation Day, PEG Concentration.

The resulted model summary indicates that the model takes into account of 64.50% variation in the parameter of parameter during process. The R^2adj (which is coefficient of determination adjusted) for the above regression equation was calculated as 59.86% (as shown above). This R^2 adj shows the proportion of variation in response explained by the regression model. So, it is found that the regression equation above is able to capture 59.86% variation in measured values of function Drug Release (Dr) for two independent process parameters within the ranges considered in study. From the Taguchi analysis it was observed that setting parameter value of Rotation Day to 2 days and Peg concentration to 1.5% yields the maximum Drug release of 86.43%. and Model equation can be used for future prediction of drug release under various parameter settings it is because the entire behaviour is captured in the model. From the above model results the formulation F18 results better drug release and selected as optimized formulation. Optimized formulations were subjected to SEM studies, FT-IR studies, Particle size, Zeta potential, and in vivo studies.

The particle size and zeta potential of the PEG Functionalized -MWCNTs- conjugate were characterized by Zeta Sizer at Poornayu research laboratories Bangalore, The particle size distribution analysis revealed that the average size of pure MWCNTs was approximately 85 nm, while the Prednisolone-loaded MWCNT formulation exhibited an increased average size of about 650 nm. This significant increase in particle size indicates the successful binding or adsorption of Prednisolone molecules onto the surface of the MWCNTs.The enlargement in particle diameter can be attributed to the formation of a drug layer around the nanotubes, resulting in a thicker and more aggregated structure. When drug molecules attach to the functionalized surfaces of MWCNTs (especially those modified with hydrophilic groups such as –COOH or –OH), they alter the surface characteristics, leading to an apparent increase in the hydrodynamic diameter measured by particle size analysis.Furthermore, the increase in size supports the evidence obtained from SEM and FTIR analyses, confirming that Prednisolone was successfully loaded onto the MWCNT surface. Thus, the observed rise in particle size from 85 nm to 650 nm serves as a strong indication of effective drug loading and complex formation between Prednisolone and MWCNTs. Furthermore, the increase in size supports the evidence obtained from SEM and FTIR analyses, confirming that Prednisolone was successfully loaded onto the MWCNT surface. Thus, the observed rise in particle size from 85 nm to 650 nm serves as a strong indication of effective drug loading and complex formation between Prednisolone and MWCNTs.

The zeta potential of the MWCNT formulation was found to be +24 mV, indicating that the particles possess a moderately high positive surface charge. This positive charge leads to electrostatic repulsion among the MWCNT particles, thereby minimizing the chances of aggregation or sedimentation. As a result, the dispersion remains physically stable over time.Thus, a zeta potential value of around ±20–30 mV is typically considered to represent a stable colloidal system, confirming that the MWCNT–drug formulation exhibits good stability and uniform distribution in suspension. Scanning Electron Microscopy (SEM) images showed noticeable changes in the morphology and an apparent increase in the diameter of the MWCNTs upon drug loading. These observations suggest successful attachment of the drug molecules onto the MWCNT surface, leading to aggregation and surface modification.The mast cell stabilizing effect of prednisolone and its MWCNT–prednisolone conjugate was investigated using the Compound 48/80–induced mast cell degranulation model in rats, a well-established experimental system for evaluating anti-allergic activity. Compound 48/80 is known to trigger mast cell degranulation by increasing intracellular calcium levels and releasing histamine and other inflammatory mediators. The pronounced degranulation observed in the control group confirmed the successful induction of allergic response.

Pretreatment with free prednisolone (5mg/kg, i.p.) significantly inhibited mast cell degranulation, which can be attributed to its corticosteroid action—stabilization of mast cell membranes, suppression of phospholipase A_2, and inhibition of the release of inflammatory mediators such as prostaglandins and leukotrienes. These results corroborate previous reports demonstrating that glucocorticoids effectively suppress allergic inflammation by preventing mast cell activation and mediator release.The MWCNT–Prednisolone conjugate (5mg/kg, i.p.) exhibited a sustained and time-dependent mast cell stabilization, with protection progressively increasing from 2 h to 24 h. This extended protection reflects the controlled release of prednisolone from the PEG-functionalized carbon nanotube matrix. The conjugate formulation provided a gradual release of drug molecules, maintaining an effective concentration for a prolonged period compared with the rapid onset but shorter duration of action of free prednisolone. The improved mast cell stabilization may also result from enhanced cellular uptake and bioavailability due to nanoscale delivery, which facilitates closer interaction of the conjugated drug with the mast cell membrane.

Summary
The aim of the present study was to prepare and evaluate the fullerene related nano carbon Prednisolone conjugates for the treatment of allergy. Functionalised MWCNT's were prepared by functionalisation of MWCNT's with PEG, Prednisolone drug binded to Functionalised MWCNT's as per DOE, prepared formulations were evaluated. FT-IR study was carried out to check the Capability of Prednisolone and PEG on MWCNTs. The major FTIR peaks of drug were retained in the FTIR of PEG Functionalized -MWCNTs- conjugate optimized formulation.,Which shows no interaction Between MWCNT and drug SEM images reveal the presence of drug on the surface of MWCNTs. Satisfactory entrapment efficiencies for all the PEG functionalised MWCNT's were obtained i.e. greater than 80% drug binded to all 27 formulations. To quantify the drug concentration in the in vitro samples, we choose UV spectrophotometric studies. Pure drug was dissolved in suitable solvent (phosphate buffer of pH 7.4: ethanol 9:1) and λ max (Wavelength) were found to be 246 nm respectively. The standard solutions were prepared using buffer Standard curve obtained with $R^2 = 0.9966$ with equation y = 0.0354x was used for the estimation of unknown samples. The PEG Functionalized - MWCNTs- conjugate optimized formulation was evaluated for particle size & zeta potential using zeta sizer. Entrapment efficiency was estimated using centrist apparatus which is able to separate aqueous phase. The in vitro dissolution studies were carried out for PEG Functionalized -MWCNTs- conjugates to find out the optimized formulation. All the formulations were able to sustain the drug release up to 24 hours The average percentage of drug released from the optimized formulation showed good sustained release up to 24 hours.
Thus, the study Summarize that the Prednisolone, especially the F18 Carbon nanotube prednisolone conjugate demonstrates significant anti-allergic efficacy. However, further detailed studies involving molecular mechanism elucidation, bioactive compound isolation and comprehensive studies are required to confirm its clinical relevance and facilitate its progression into a safe therapeutic candidate and effective phytopharmaceutical agent for allergic disease.

CONCLUSION
MWCNTs are progressive nanocarrier for the delivery of antiallergy drug Prednisolone. Surface modification of MWCNTs was done by non-covalent functionalization using polyethylene glycol (PEG). Different formulations were designed using MINITAB software version 18.0 by binding Antiallergy drug to different PEG functionalized MWCNTs. Design of experiments were done by Taguchi method. 27 formulations were designed by binding to Prednisolone PEG Functionalized MWCNTs. All the formulations were evaluated for drug release and drug entrapment studies. Optimized formulation was subjected to SEM, FT-IR, Particle size, Zeta potential, and In vivo studies. The results of the in vivo study demonstrated that the prednisolone significantly stabilized mast cells against Compound 48/80–induced degranulation in rat mesentery preparations. Among the Carbon nanotubes prednisolone conjugates tested, the Carbon nanotubes prednisolone conjugates exhibited the highest mast cell protective effect, The in vivo studies further validated these findings, wherein pre-treatment with the active carbon nanotubes prednisolone conjugates significantly reduced mast cell degranulation in experimental animals. Collectively, these findings provide scientific evidence to support prednisolone in the management of allergic disorders. The observed mast cell stabilizing anti-allergic activities may be attributed to the synergistic action of its bioactive constituents' release of chemical mediators of allergy. Thus, the study concludes that the Prednisolone, especially the F18, demonstrates significant anti-allergic efficacy. However, further detailed studies involving molecular mechanism elucidation, bioactive compound isolation and comprehensive studies are required to confirm its clinical relevance and facilitate its progression into a safe therapeutic candidate and effective phytopharmaceutical agent for all allergic disease.

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