The Bioscan
AN INTERNATIONAL QUARTERLY JOURNAL OF LIFE SCIENCES
20(4): 471-484, 2025 www.thebioscan.com

Preparation and Evaluation of Fullerene Related Nano Carbon Phenobarbitone Conjugates for the Treatment of Epilepsy

Nanditha K[1], Hemalatha KP*[2], Vijaykumar S[3], Mancy SP[4]

[1]Department of Pharmaceutics, Sree Siddaganga College of Pharmacy, Tumakuru - 572103, Karnataka, India
[2]Assistant Professor, Department of Pharmaceutics, Sree Siddaganga College of Pharmacy, Tumakuru-572103, Karnataka, India
[3]Assistant Professor, Department of Pharmaceutics, Sree Siddaganga College of Pharmacy, Tumakuru-572103, Karnataka, India
[4]Associate Professor, Department of Pharmacology, Sree Siddaganga College of Pharmacy, Tumakuru-572103, Karnataka, India

DOI: 10.63001/tbs.2025.v20.i04.pp471-484

KEYWORDS:
Polyethylene glycol, Multi Walled Carbon Nanaotubes, Design of Experiments, Scanning Electron Microscopy, Maximal Electroshock Seizure, Epilepsy

Received on:

06-10-2025

Accepted on:

13-11-2025

Published on:

19-11-2025

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 Phenobarbitone. The primary goal was to create a PEG–MWCNT–Phenobarbitone 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 Phenobarbitone 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 95 to 950 nm, and a zeta potential of 23 mV indicated good stability of the formulation. The drug entrapment efficiency exceeded 85%, 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 optimized formulation demonstrated notable anticonvulsant activity in the Maximal Electroshock Seizure (MES) model, reflected by a significant reduction in the tonic limb extension phase. Statistical analysis validated the consistency and reliability of the experimental data. Overall, the PEG-functionalized MWCNT–Phenobarbitone conjugate showed excellent dispersion, stability, and prolonged release characteristics, indicating its strong potential as an advanced drug delivery system for epilepsy therapy. This study highlights the promising role of functionalized carbon nanotubes in improving the pharmacological performance of conventional antiepileptic drugs.

1.INTRODUCTION

Carbon nanotubes (CNTs) were first observed and described in 1952 by Radushkevich and Lukyanovich and later in 1976, the single or double walled carbon nanotubes were observed. However in modern history, the discovery of CNTs is largely attributed to Sumio Iijima (1991) as the first scientist who described the multi walled carbon nanotubes (MWCNTs)[1]. CNT are allotropes of carbon consisting of a one-atom thick sheet of graphite (called graphene) rolled up seamlessly into a cylindrical structure with diameter in the nanometer range.

Carbon nanotubes are also called buckytubes, possess unique thermal, electrical and mechanical properties which make them useful in a wide range of applications. Structurally, CNTs have fullerene like configuration composed of graphene sheets where each carbon atom is sp2 hybridized[2]. CNTs exhibit extremely high aspect ratios (length to diameter ratio), typically greater than 1000 and sometimes reaching upto 2,50,000, which render their nanostructure quasi-one-dimensional (1D) [3,4] . The nanotube is cylindrical with at least one end typically capped with a hemispherical configuration[5].

MWCNTs have a high surface area and a hallow, nanoscale tubular structure that enables loading of substantial quantities of different typesof drugs both hydrophilic and hydrophobic. [6]. The larger the inner cavity and the outer walls can be used for both physical adsorption(via π-π stacking, hydrophobic interactions) and chemical conjugation, supporting multilayer loading[7] .

Functionalized carbon Nanotubes (f-CNTs) have emerged as new tools in the field of nanobiotechnology and nanomedicine. CNTs are the long, hollow, seamless cylinders of graphene which exists as single walled or multiwalled carbon nanotubes structures, with diameter typically ranging from 1-100 nm [8] . Structurally, CNTs are hollow cylinders of sp2 hybridized carbon atoms[9]. The

bonding structure, composed of strong sp^2 carbon–carbon bonds, provides CNTs can interlink through partial conversion of sp^2 to sp^3 bonds potentially forming ultra-strong continuous carbon wires [10] .

CNTs are hollow nanostructured materials consisting of carbon atoms bonded to three neighbor atoms via sp^2 hybridization, which contributes to their remarkable strength. CNTs represent a nanocrystalline carbon clusters structurally built from graphene sheets rolled into a tube that is closed at the ends by the fullerene caps [11]. Depending on synthesis conditions, nanotubes can be produced in a single, double or multi-walled arrangement. CNTs often self-assemble into bundles containing several to hundreds of tubes held together by van der Waals forces [12]. CNTs have the unique properties of nano-size, high aspect ratio, strong mechanical strength and electric conductivity, therefore CNTs have attracted significant interest across multiple fields – particularly in electronics, energy devices and biomedical sciences [13].

Functionalized MWCNTs can effectively deliver antiepileptic drugs such as phenobarbitone, carbamazepine, and valproic acid in a controlled and sustained manner, minimizing fluctuations in plasma concentration and enhancing therapeutic efficacy.[14] Their surface modification with biopolymers like polyethylene glycol (PEG) improves solubility and reduces toxicity, ensuring better brain targeting and prolonged drug action. Additionally, MWCNTs have been explored for neuronal interfacing and brain tissue engineering, offering potential applications in neural modulation and seizure control through electrical stimulation and drug–nanotube conjugates. [15]

Phenobarbitone is a long-acting barbiturate widely used for the treatment of epilepsy and seizure disorders. Despite its therapeutic efficacy and prolonged half-life, conventional phenobarbitone formulations present several disadvantages, including variable bioavailability, dose-dependent toxicity, sedation, and reduced patient compliance due to frequent dosing requirements. Moreover, sustained high plasma concentrations can lead to tolerance and dependence with long-term therapy. [16]

To overcome the limitations of conventional phenobarbitone therapy, the drug was incorporated into polyethylene glycol (PEG)-functionalized multi-walled carbon nanotubes (MWCNTs). MWCNTs provide a unique nanoscale hollow structure and high surface area, enabling efficient drug loading, while PEG functionalization enhances their aqueous dispersibility, biocompatibility, and stability within biological environments. This nanocarrier system is designed to achieve controlled and sustained drug release, thereby minimizing dosing frequency and reducing adverse effects associated with conventional administration.

Furthermore, MWCNTs can promote improved cellular uptake through mechanisms such as endocytosis and enhanced membrane permeability, facilitating more effective transport of phenobarbitone to target sites. The gradual and continuous release of the drug from MWCNTs helps maintain therapeutic plasma concentrations for prolonged durations, ensuring stable drug levels and preventing the fluctuations that often lead to side effects or subtherapeutic responses in traditional dosing regimens.

2.MATERIALS

The following drug were obtained from commercial suppliers and used as received: Phenobarbitone (Harman finochem limited medopharm,Guduvanchery), Carbon nano tubes (Nano wings pvt. Ltd Hyderabad).

3.METHODS

FUNCTIONALIZATION OF MWCNT's [17]

Prepare 100 mg/ml PEG solution in distilled water. Accurately weigh 100 mg of pristine MWCNTs in a beaker and add 10 ml of prepared PEG solution. Sonicate the mixture for 30 minutes in Utrasonic Bath sonicator to ensure uniform dispersion. After sonication transfer the sonicated solution into centrifuge tube and centrifuge at 3000 rpm to remove the unbound or agglomerated PEG from MWCNTs. Carefully decant the supernatant and collect the sediment (MWCNTs dispersion). Wash the obtained material several times with distilled water to eliminate residual PEG. Finally, clarify the dispersion using a 0.2µm Millipore vacuum filter and dry the filtered MWCNTs.

Experimental Design and Optimization of Phenobarbitone Loading on PEG Functionalized MWCNTs [18, 19]

To optimize the key formulation parameters influencing drug loading, the Taguchi Design of Experiments (DoE) approach was employed using Minitab 18 software. This statistical design method helps identify the most significant factors and their optimal levels with a minimal number of experimental runs, thereby improving efficiency and reproducibility.

Two independent variables were selected for the study: Factor A - Percentage of PEG functionalization (5%, 10%, and 15%), Factor B- Rotation time (1 day, 2 days, and 3 days) The response variable was the drug entrapment efficiency (%) of phenobarbitone in PEG-MWCNTs.

According to Taguchi's orthogonal array design, the total degrees of freedom (DOF) were calculated as follows:

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

i.e, (3 - 1) 2 + (3-1)(3-1) = 8.

Therefore, the minimum number of experiments required was DOF + 1 =9. Considering the need to study main and interaction effects comprehensively an L27 orthogonal array was selected.

The L27 orthogonal array was constructed in Minitab software, generating matrix comprising all combinations of the two factors at three levels. Signal-to-noise (S/N) ratios were computed using the "larger-the-better" criterion to maximize entrapment efficiency. Analysis of variance (ANOVA) determined the relative contribution of each factor and their interactions on the response. The optimized formulation will be identified based on the highest S/N ratio and subsequently validated through experimental trails.

This systematic Taguchi design approach ensured the identification of significant formulation parameters with reduced experimental trials, leading to a more robust and reproducible phenobarbitone-loaded PEG-MWCNT system.

Phenobarbitone-loaded PEG functionalized multi-walled carbon nanotubes (PEG-MWCNTs) were prepared by mixing PEG functionalized MWCNTs (5%, 10%, and 15%) and phenobarbitone in a 1:1 ratio. The mixture was sonicated for 30 minutes to achieve uniform dispersion, followed by rotation on an orbital shaker for 1, 2 & 3 days to facilitate the drug loading onto the functionalized MWCNTs. The resulting mixture was dried at room temperature for 24 hours, yielding the PEG-MWCNT-Phenobarbitone conjugate (PEG-MWCNT-PB), which was stored in a vacuum desiccator until further analysis.

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

Experimental run	Drug conc (mg)	Binding time (days)	PEG 6000 Fun- MWCNT's
F1	5	1	5 %
F2	5	1	10%
F3	5	1	15%
F4	5	2	5 %
F5	5	2	10%
F6	5	2	15%
F7	5	3	5 %
F8	5	3	10%
F9	5	3	15%
F10	5	1	5 %
F11	5	1	10%
F12	5	1	15%
F13	5	2	5 %
F14	5	2	10%
F15	5	2	15%
F16	5	3	5 %
F17	5	3	10%
F18	5	3	15%
F19	5	1	5 %
F20	5	1	10%
F21	5	1	15%
F22	5	2	5 %
F23	5	2	10%
F24	5	2	15%
F25	5	3	5 %
F26	5	3	10%
F27	5	3	15%

Evaluation of PEG Functionalized MWCNTs Phenobarbitone Conjugate

Drug entrapment efficiency[20]

Drug entrapment efficiency was assessed by dispersing 5 mg of PEG-MWCNT-PB complex in 10 mL phosphate buffer (pH 7.4) and heat to 37 °C. The sample was centrifuged at 5000 rpm for 1 hour, and the supernatant was diluted appropriately. Phenobarbitone entrapment was quantified using a UV spectrophotometer at 238 nm to determine the drug loading.

In vitro release studies [21]

The cumulative drug release of all formulations was assessed using a dialysis membrane (HIMEDIA-70) with phosphate buffer (pH 7.4) as the dissolution medium. For each test, 5mg of PEG-MWCNT-PB was placed into a pre-soaked dialysis tube, securely sealed, and immersed in 100 mL of buffer at 37 °C in a shaking water bath at 50 rpm. At predetermined intervals, 1 mL samples were withdrawn and immediately replaced with an equal volume of fresh buffer to maintain sink conditions. Withdrawn samples were diluted as necessary and analyzed by UV-Vis spectrophotometry at 238 nm to determine Phenobarbitone release. The cumulative drug release (%CDR) was calculated using:

Particle size was measured by Dynamic Light Scattering (DLS). The sample dispersion was well-shaken to break aggregates, and 1 mL was transferred into a clean disposable cuvette. Measurements were taken using right-angle light scattering, and data were processed through cumulant analysis after minimizing noise from dust and impurities.

Zeta potential [24]

Zeta potential was measured to assess the colloidal stability of functionalized CNTs. The dispersion was sonicated at 90 Hz for 15 minutes and kept at room temperature for 24 hours. Then, 1 mL of the sample was transferred into a disposable folded capillary cuvette, and measurements were performed using a zeta potential analyzer.

SEM Studies [25]

Scanning Electron Microscopy (SEM) was used to study the surface morphology of the samples. The dispersion was sonicated to break agglomerates, and a drop was placed on an SEM grid and air-dried. The dried sample was examined under vacuum (~1×10^{-3} Pa) using an electron beam, and backscattered electrons were detected to visualize the surface structure at nanometer resolution.

In vivo release studies[25,26]

Investigation of anti-epileptic activity by Maximal electro shock (MES)-induced convulsions.

GROUPING OF ANIMALS

$$\%\ \mathbf{CDR} = (\text{Total loaded drug} - \text{each time interval released drug} + \text{Previous total released drug})/(\text{Total loaded drug}) * 100$$

FT-IR Studies [22]

Fourier Transform Infrared (FTIR) spectroscopy was performed to record the absorption spectra of the samples. About 5 mg of the sample was blended with 100 mg of potassium bromide (KBr) and compressed into a transparent pellet. The pellet was placed in a clean sample holder, and spectra were scanned in the range of 400–4000 cm^{-1}.

Particle size [23]

Table no. 2 Details of treatment on different animal groups

The animals will be divided into 4 groups (n = 6)

GROUPS	DESCRIPTION	TREATMENT
GROUP 1	MES control	Normal saline (10 ml / kg.b.wt.)
GROUP 2	Formulation group	Phenobarbitone conjugate (4 mg / kg.b.wt.)
GROUP 3	Marketed group	(4 mg / kg.b.wt.)
GROUP 4	Standard group	(4 mg / kg.b.wt.)

PROCEDURE

Healthy Albino *Wistar* rats of either sex, weighing 180-200 g were used in the present study. The animals were housed under standard laboratory conditions, including regulated temperature, a 12-hour light/dark cycle, and constant access to standard chow and water ad libitum. Each group was pre-treated with either the test formulation or standard drug at the designated doses for four consecutive days. On the fifth day, one hour after administration of the test drug or standard, the rats were subjected to maximal electroshock (MES) to induce seizures. Following induction, the distinct phases and duration of epileptic episodes were carefully observed and documented. The percentage protection against MES-induced seizures was

subsequently calculated and compared across groups to assess anticonvulsant efficacy relative to the standard drug.

4.RESULTS

Table no 3 Drug Entrapment Results

Formulation Code	Drug Entrapment efficiency %	Formulation Code	Drug Entrapment efficiency %
F1	60 .25	F15	78.54
F2	78 .45	F16	82.06
F3	75.55	F17	76.44
F4	77.67	F18	72.39
F5	82.54	F19	70.24
F6	79.44	F20	83.45
F7	84.38	F21	71.39
F8	78.04	F22	73.06
F9	79.68	F23	75.09
F10	80.67	F24	80±28
F11	79.28	F25	80.67
F12	81.43	F26	77.08
F13	85.68	F27	74.35
F14	79.62	F22	77±0.43
F9	80±0.76	F23	78± 0.61
F10	83±0.27	F24	75.3±0.7
F11	80.2±0.49	F25	76±0.72
F12	86.3±0.59	F26	75.3±0.43
F13	83±0.79	F27	77±0.8
F14	84.2±0.25		

Table no. 4 In vitro release studies

Formulation Code	% CDR	Formulation Code	% CDR
F6	79.44	F20	83.45
F7	80±0.65	F21	87±0.65
F8	82±0.75	F22	86±0.69
F9	85±0.2	F23	84±0.55
F10	84±0.76	F24	76±0.35
F11	83.2±0.47	F25	78±0.5
F12	82±0.25	F26	76±0.34
F13	87±0.68	F27	75±0.5
F14	79±0.48	F22	77±0.43
F9	80±0.76	F23	78± 0.61
F10	83±0.27	F24	75.3±0.7
F11	80.2±0.49	F25	76±0.72
F12	86.3±0.59	F26	75.3±0.43
F13	83±0.79	F27	77±0.8
F14	84.2±0.25		

Table no 5: Experimental results with computed S/N ratio

Expt. No.	Parameters		Drug Release (Dr)				S/N ratio
	Rotation Day 'R'	PEG '%'	Trial #1	Trial #2	Trial #3	Mean	
1	1	0.5	80	82	85	82.3333	38.3035
2	1	1.0	81	83	82	82.0000	38.2750
3	1	1.5	85	79	80	81.3333	38.1922
4	2	0.5	83	80	86	83.0000	38.3702
5	**2**	**1.0**	**83**	**84**	**87**	**84.6667**	**38.5491**
6	2	1.5	86	84	76	82.0000	38.2385
7	3	0.5	70	76	75	73.6667	37.3284
8	3	1.0	77	78	75	76.6667	37.6887
9	3	1.5	76	75	77	76.0000	37.6148

Table no. 6 Response Table for S/N Ratios (Larger is better)

Level	Rot. Days	PEG.Conc
1	38.26	38.00
2	38.39	38.17
3	37.54	38.02
Delta	0.84	0.17
Rank	1	2

Table No. 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 No. 8 Regression Co-efficient of the Model

R-sq	R-sq(adj)
38.62%	33.50%

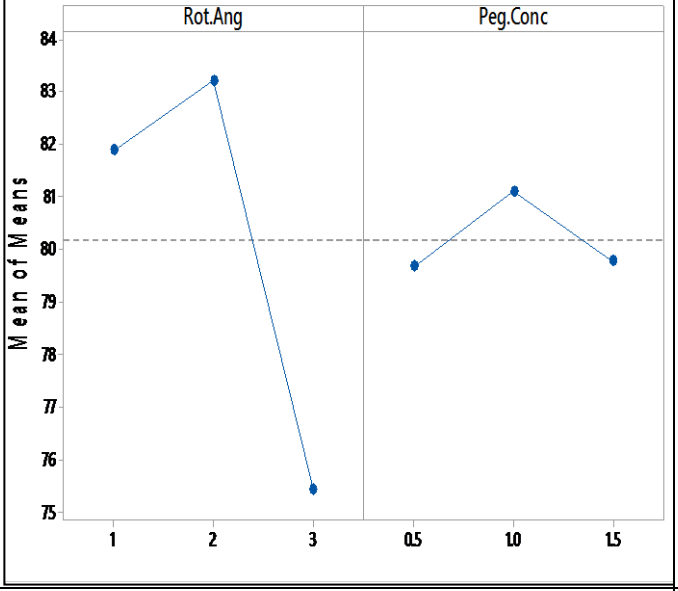


Fig No. 1 Main effect plot for mean

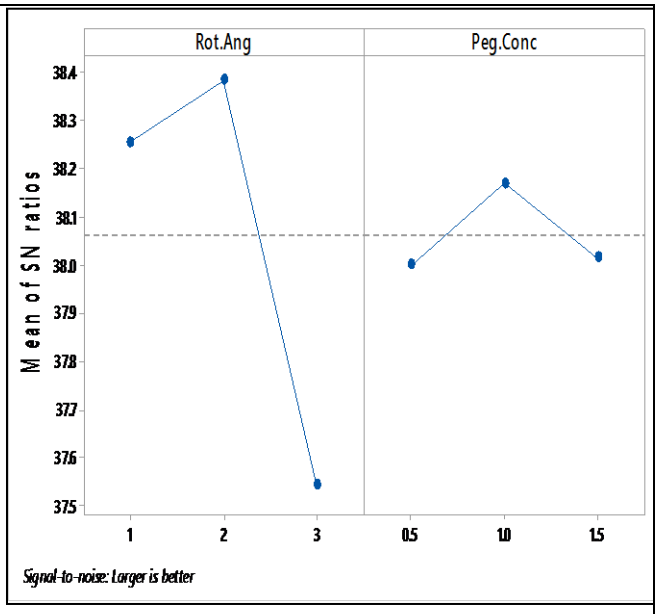


Fig No . 2 Main effect plot for S/N ratio

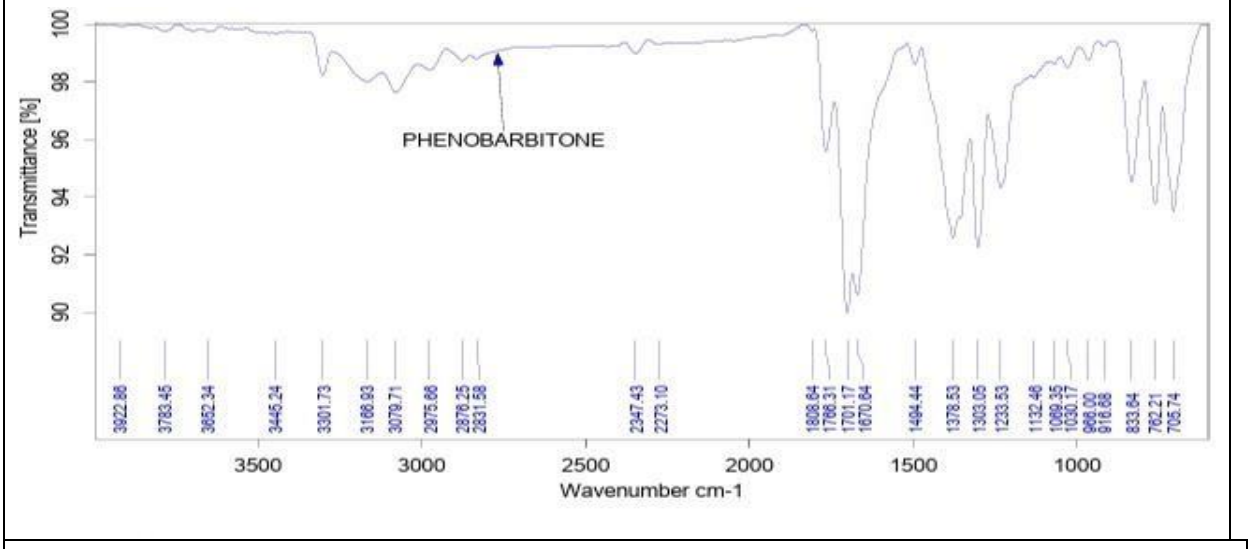


Fig No. 3 FT-IR Spectrum of Phenobarbitone

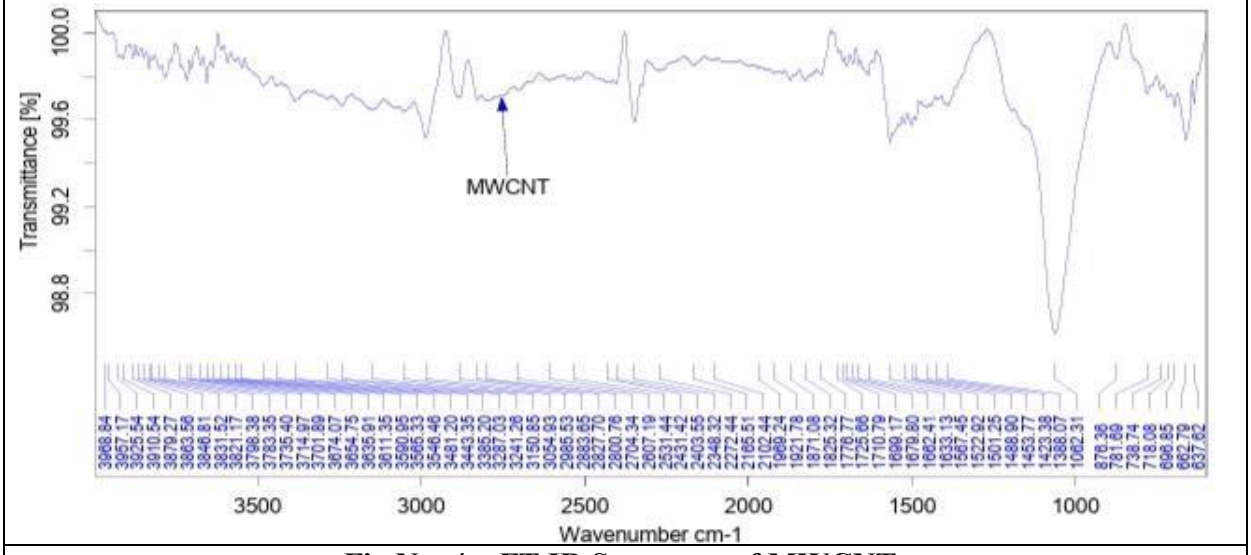


Fig No. 4 FT-IR Spectrum of MWCNTs

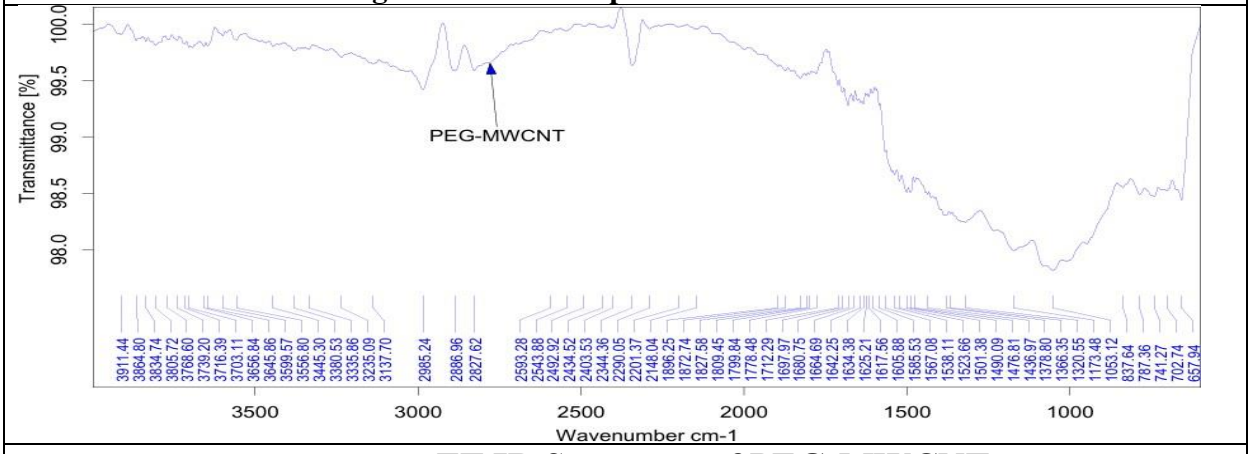


Fig No.5 **FT-IR Spectrum of PEG-MWCNTs**

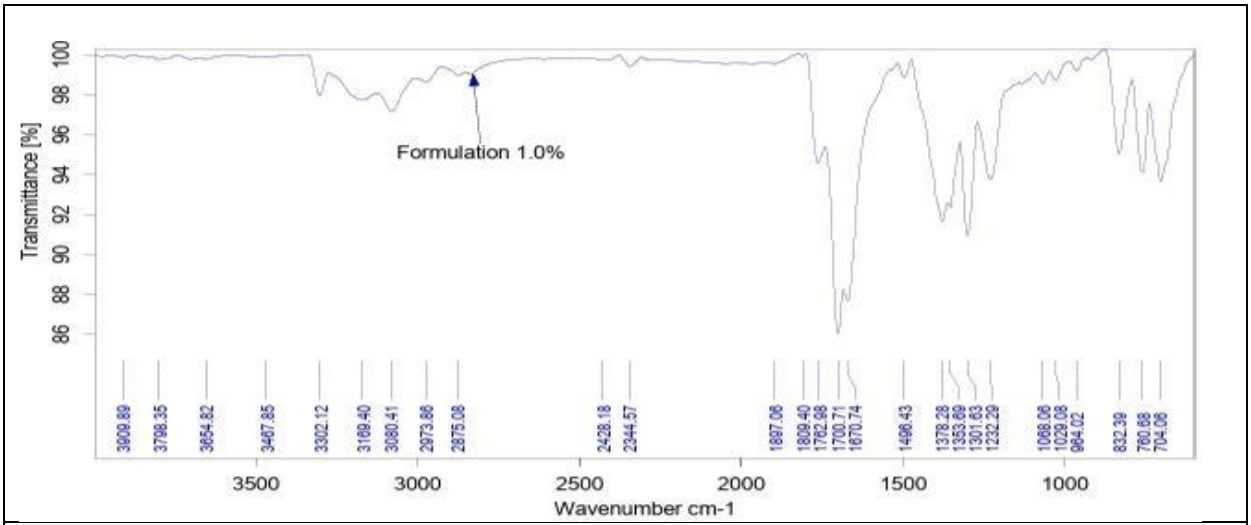


Fig No. 6 **FT-IR Spectrum of optimized formulation**

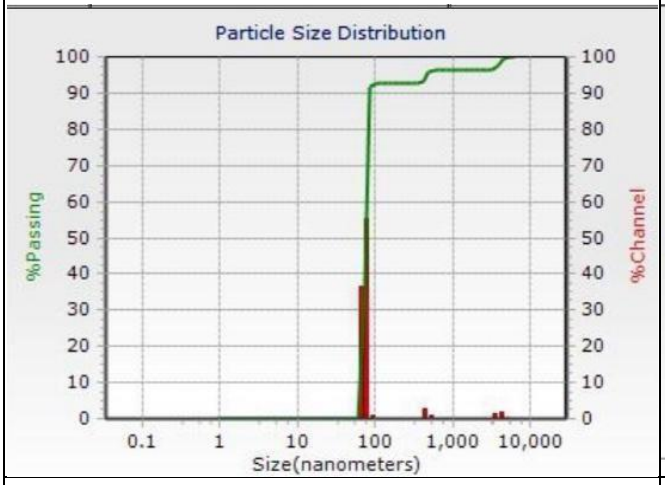


Fig no .7 Size distribution profile of MWCNTs

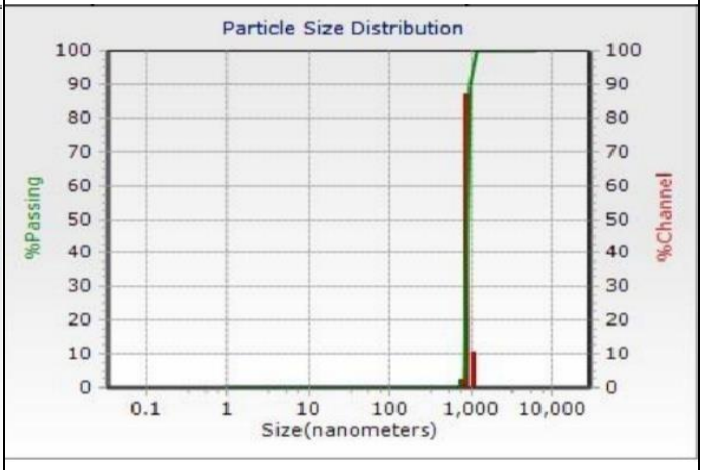


Fig no. 8 Size distribution profile of optimized formulation

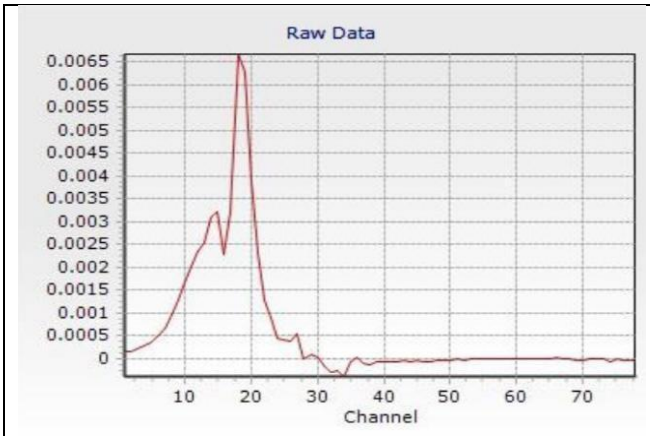


Fig no 9 Zeta potential of MWCNTs

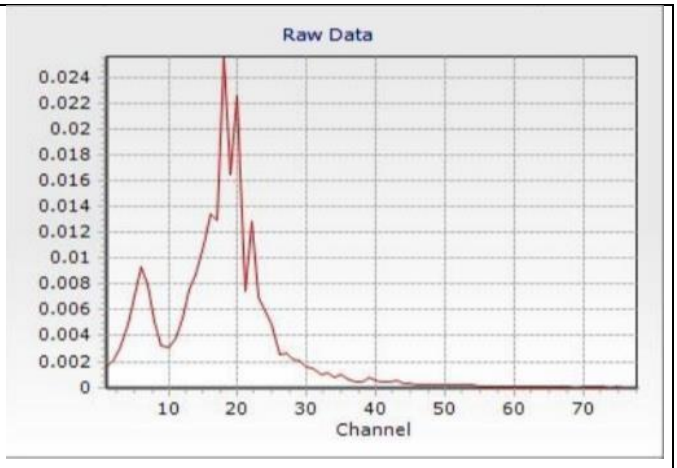


Fig no 10 **Zeta potential of optimized formulation**

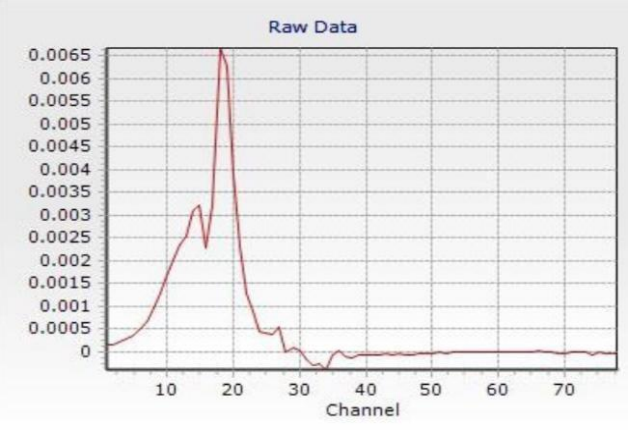


Fig no 9 Zeta potential of MWCNTs

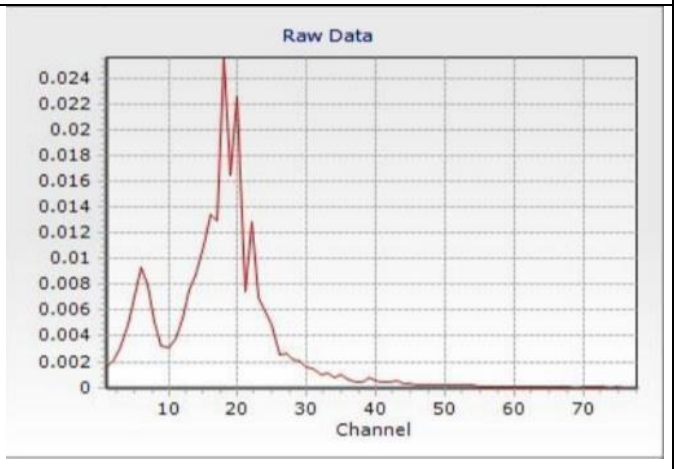


Fig no 10 **Zeta potential of optimized formulation**

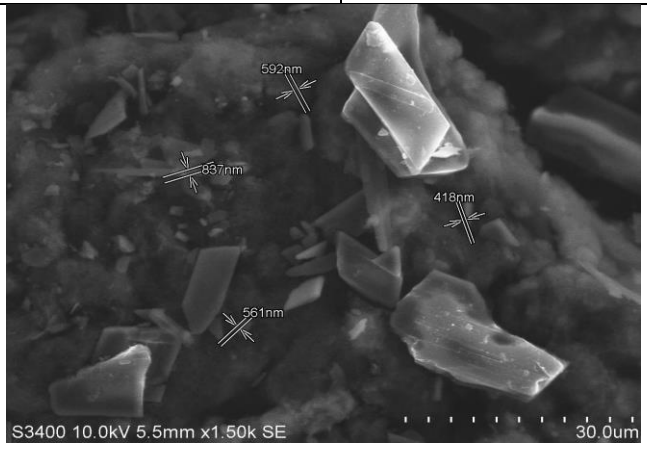


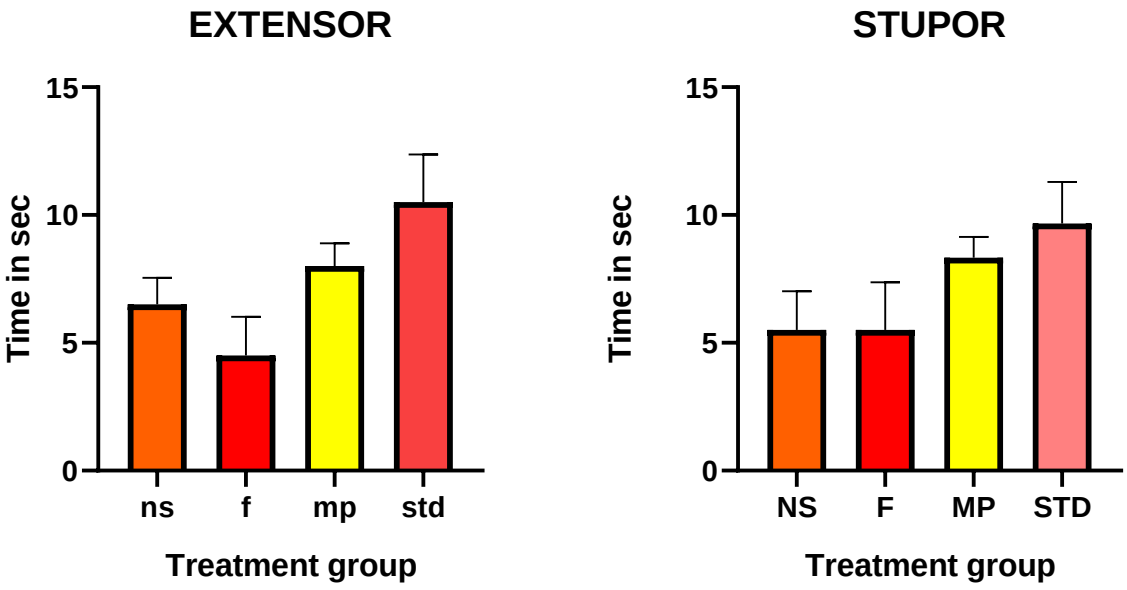
Fig No 11 **SEM of optimized formulation**

In vivo studies: Effect of anti-epileptic activity of Phenobarbitone

Table no 9: Effect of Phenobarbitone on MES-induced convulsion on rats

Group	Treatment	Various phases of convulsion(sec)		
		Extension	Stupor	Recovery/Death
1	MES-Control	6.50±0.42	5.50±0.61	R
2	Formulation	9.66±0.68	5.50±0.76	R
3	Marketed	6.66±0.66	8.33±0.33*	R
4	Standard	10.50±0.76***	9.66±0.66***	R

Values are expressed as Mean ± SEM (n-6) by one way ANOVA followed by Tukey test. Where,* represent significant at p<0.05, ** represent significant at p<0.01, *** represent significant at p<0.001, **** represent significant at p<0.0001, compared to the MES-Control.



5.DISCUSSION

The design of experiments (DOE) was carried out using the Taguchi method with the statistical software tool MINITAB 18.0. In this research, pristine multi-walled carbon nanotubes (MWCNTs) were utilized for evaluating their potential in antiepileptic activity. However, synthetically produced MWCNTs often contain metallic and amorphous carbon impurities and exhibit poor water solubility, which limit their biomedical applications. Moreover, the weak interfacial and van der Waals interactions between CNTs and polymer matrices further restrict their dispersion and functional performance. To overcome these limitations, surface modification of CNTs has been explored, particularly through non-covalent functionalization methods. In this study, polyethylene glycol (PEG) functionalized MWCNTs were prepared via a non-covalent approach to enhance solubility, stability, and biocompatibility for efficient drug delivery in antiepileptic therapy. The PEG-functionalized multi-walled carbon nanotubes (MWCNTs) were loaded with the biocompatible antiepileptic drug Phenobarbitone to develop a sustained and targeted drug delivery system for epilepsy. Functionalization with polyethylene glycol (PEG) improved the aqueous dispersibility, stability, and biocompatibility of MWCNTs, enabling efficient drug adsorption through non-covalent interactions such as π-π stacking, hydrogen bonding, and van der Waals forces. The PEG coating also minimized cytotoxicity and prevented aggregation of nanotubes, ensuring safe interaction with biological systems. Phenobarbitone, a conventional antiepileptic agent, was chosen due to its established therapeutic efficacy and long half-life, which complements the slow and controlled release properties of the PEG-MWCNT matrix. This formulation is designed to enhance drug retention, facilitate brain targeting, and reduce dosing frequency, thereby improving patient compliance and therapeutic outcomes in epilepsy management. PEG Functionalized-MWCNTs-conjugates were prepared according to

DOE given in table 1 using Taguchi statistical method in MINITAB software. All the prepared formulations were subjected to in vitro drug release and entrapment efficiency studies. The synthesized PEG-functionalized MWCNT–Phenobarbitone conjugates exhibited excellent drug Entrapment, ranging from 60.25% to 84.62%. The in vitro drug release studies were performed at different pH conditions and time intervals to evaluate the release behavior of the PEG-functionalized MWCNT–Phenobarbitone formulations. The experiments designed using Taguchi's L27 Orthogonal Array (OA) to systematically study the influence of selected formulation variables on the release profile. while the corresponding drug release data (response variable) obtained from the studies were statistically analyzed using the MINITAB 17.0 software, as summarized in Table 3. The statistical evaluation helped identify the most significant factors influencing drug release and facilitated the selection of the optimized formulation based on the signal-to-noise (S/N) ratio and analysis of variance (ANOVA) results. The results presented in Table 6 indicate that the maximum signal-to-noise (S/N) ratio was obtained at Rotation Days – Level 2 and PEG Concentration – Level 1. Similarly, Table 7 shows that the maximum mean drug release also occurred at the same settings, confirming these as the optimal process parameters. Furthermore, Table 12 demonstrates that Rotation Days had the minimum rank value and was assigned Rank 1, while PEG Concentration was ranked 2, reinforcing that Rotation Days exerting the greatest influence on drug release. Thus, the software identified the optimized formulation corresponding to Rotation Days = 2 and PEG Concentration = 1.

The Analysis of Variance (ANOVA) data in Table 7 revealed that Rotation Days contributed 38.6% (186.889/484.074)×100(186.889/484.074) × 100(186.889/484.074)×100 to the total variation in the response, while PEG Concentration contributed 1.2% (0.056/484.074)×100(0.056/484.074) × 100(0.056/484.074)×100. This clearly indicates that Rotation Days is the most significant factor influencing the S/N ratio and, consequently, the drug release , while PEG concentration has a comparatively minor effect. The higher F-value observed for Rotation Days further confirms its stronger effect on drug release. The analysis was conducted at a significance level (α) = 0.05, corresponding to a 95% confidence level. The ANOVA table also showed that the P-value < 0.05 for Rotation Days, establishing its statistical significance in affecting the drug release behaviour. A regression model was developed using MINITAB 18.0, taking Drug Release as the response variable and Rotation Days and PEG Concentration as independent process parameters. The model summary indicated that the regression equation accounted for 38.6% of the total variation, with an adjusted R^2 (R^2adj) value of 33.5%. This demonstrates that the model was able to explain 33.5% of the variation in the observed drug release response within the range of experimental conditions studied.

The optimized formulation was identified Formulations no 14 using the signal-to-noise (S/N) ratio generated by the Taguchi design in MINITAB software, based on the response variable of drug release for all tested formulations. The effects parameters on the Drug Release were analyzed using MINITAB R18.0 Statistical Software and the results are shown in Figure 1 and 2, Fig 1 indicates a there is a maximum SN ratio for setting Rot Angle at 2 and Peg concentration at one. Also Fig 2 indicates a there is a maximum mean drug release for setting Rot Angle at 2 and Peg concentration at one. The optimized sample was further characterized using Scanning Electron Microscopy (SEM) to analyze surface morphology, Fourier Transform Infrared Spectroscopy (FT-IR) to confirm functional group interactions, particle size and zeta potential analysis to determine dispersion stability, and finally, in vivo studies to evaluate the therapeutic efficacy and biocompatibility of the formulation in antiepileptic activity. The FT-IR spectrum of the optimized formulation depicted in figure 6 shows characteristic peaks confirming the presence and compatibility of all components by comparing the peaks from figure 3, 4, and 5. The N–H stretching band observed between 3500–3100 cm^{-1} indicates the presence of amine groups from phenobarbitone. The C–H stretching bands above 2850 cm^{-1} and around 2900–2960 cm^{-1} correspond to aliphatic and aromatic C–H vibrations, confirming the structural integrity of the drug and carrier. The C=O stretching band signifies the carbonyl group of phenobarbitone, while the C–O–C stretching band at 1100–1150 cm^{-1} confirms the presence of ether linkages from PEG-functionalized MWCNTs. A broad O–H stretching band around 3400–3500 cm^{-1} indicates hydrogen bonding and the presence of hydroxyl groups from PEG. The absence of any significant deviation or shift in characteristic peaks suggests no chemical interaction between the drug and excipients, confirming the compatibility and stability of the optimized formulation. The particle size distribution of pristine MWCNTs and optimized PEG-functionalized MWCNT–Phenobarbitone formulation was found to be 95 and 950 nm depicted in the figure 7 and 8 respectively, indicating a moderately polydisperse system. After loading with Phenobarbitone, increase in particle size was observed, which can be attributed to the successful adsorption or surface binding of the drug molecules onto the PEG-functionalized MWCNT surface. This increase confirms effective drug loading and formation. The measured zeta potentials are +18 mV for pristine MWCNTs and +23 mV for the PEG-functionalized MWCNT–Phenobarbitone conjugate depicted in figure 9 and 10. Both values lie in the range typically described as moderate colloidal stability, but the PEG-functionalized conjugate shows a small positive shift relative to the unmodified nanotubes, it shows Improved electrostatic repulsion due to +5 mV increase indicates a slightly higher net surface charge after PEGylation and drug loading, which increases electrostatic repulsion between particles and helps reduce immediate aggregation. Steric stabilization from PEG Beyond charge, PEG provides *steric* (physical) barriers that prevent close approach of particles. Even a modest increase in zeta potential combined with PEG's steric effect produces better overall dispersion stability than the zeta numbers alone imply and Confirmation of surface modification and loading The change in surface potential supports that PEG functionalization and Phenobarbitone binding altered the nanotube surface chemistry — consistent with successful conjugation. The SEM images (Figure 11) revealed a noticeable increase in the dimensions and morphological variations of the optimized PEG-functionalized MWCNT–Phenobarbitone formulation, with particle sizes ranging from 418 to 837 nm. This increase in size compared to pristine MWCNTs confirms the successful adsorption of Phenobarbitone molecules onto the nanotube surface. The observed fibrillar appearance is comparable to natural fibrous materials, indicating uniform coating and stable drug attachment. The morphological enlargement further supports effective drug loading and surface modification, which contribute to improved dispersion, stability, and sustained drug release properties of the formulation. The data represent the effect of different treatments on various phases of convulsion in the Maximal Electroshock Seizure (MES) model. The formulation group showed an increase in the extension phase (9.66 ± 0.68 s) compared to the control (6.50 ± 0.42 s), indicating improved anticonvulsant activity, while the stupor phase remained comparable to the control. The marketed formulation exhibited a moderate increase in stupor duration ($p < 0.05$), whereas the standard drug showed a highly significant ($p < 0.001$) prolongation of both the extension and stupor phases depicted in table 9 and Figure 12, demonstrating maximum protection against seizures. All treated animals recovered without mortality. Statistical analysis using one-way ANOVA followed by Tukey's test confirmed the significance levels, indicating that the PEG-functionalized MWCNT–Phenobarbitone formulation

possesses notable antiepileptic potential, approaching that of the standard treatment.

6.SUMMARY

The study employed the Taguchi Design of Experiments (DOE) using MINITAB 18.0 to optimize the formulation of polyethylene glycol (PEG)-functionalized multi-walled carbon nanotubes (MWCNTs) for sustained delivery of the antiepileptic drug Phenobarbitone. Pristine MWCNTs were first functionalized with PEG via a non-covalent approach to improve their solubility, stability, biocompatibility, and dispersion, thereby overcoming issues of poor aqueous solubility and agglomeration commonly associated with raw nanotubes. PEG coating provided steric stabilization, minimized cytotoxicity, and enhanced drug adsorption through π–π stacking, hydrogen bonding, and van der Waals interactions.

The Taguchi L27 orthogonal array was used to evaluate the influence of rotation days and PEG concentration on drug entrapment and release behavior. Drug entrapment ranged from 60.25% to 84.62%, with the highest drug release and signal-to-noise (S/N) ratio observed at Rotation Days = 2 and PEG Concentration = 1, confirming these as the optimal process parameters. ANOVA analysis revealed that Rotation Days had a significant effect ($p < 0.05$) on drug release, contributing 38.6% to the total variation, whereas PEG concentration contributed only 1.2%. The developed regression model explained 33.5% (R^2adj) of the variability in drug release, validating the experimental optimization.

Characterization of the optimized formulation confirmed successful drug loading and surface modification. FT-IR spectra showed characteristic peaks for N–H, C–H, C=O, and C–O–C groups without any major shifts, indicating no chemical interaction and good compatibility between drug and excipients. Particle size increased from 95 nm (pristine MWCNTs) to 950 nm after PEG functionalization and drug loading, while zeta potential shifted from +20 mV to +23 mV, demonstrating enhanced dispersion stability. SEM images revealed uniform coating and fibrillar morphology, confirming effective PEGylation and Phenobarbitone binding.

In in vivo antiepileptic studies using the Maximal Electroshock Seizure (MES) model, the optimized PEG-MWCNT–Phenobarbitone formulation significantly prolonged the extension phase (9.66 ± 0.68 s) compared to the control (6.50 ± 0.42 s), indicating enhanced anticonvulsant efficacy. All treated animals recovered without mortality. Statistical evaluation (ANOVA followed by Tukey's test) confirmed that the PEG-functionalized formulation showed notable therapeutic potential, closely approaching that of the standard drug, thereby demonstrating its promise as a sustained and targeted antiepileptic drug delivery system.

7.CONCLUSION

The present study successfully developed and optimized a PEG-functionalized multi-walled carbon nanotube (MWCNT)–Phenobarbitone conjugate using the Taguchi Design of Experiments (DOE) approach in MINITAB 18.0 to achieve an effective sustained and targeted antiepileptic drug delivery system. Non-covalent PEG functionalization significantly improved the solubility, dispersibility, stability, and biocompatibility of MWCNTs, while facilitating efficient Phenobarbitone loading through π–π interactions, hydrogen bonding, and van der Waals forces.

Statistical optimization revealed that Rotation Days was the most influential factor on drug release, contributing 38.6% of the total variation, whereas PEG concentration had a lesser effect. The optimized formulation (Rotation Days = 2, PEG Concentration = 1) demonstrated high entrapment efficiency (up to 84.62%) and controlled in vitro release, confirming the effectiveness of the Taguchi model in process optimization.

Characterization through FT-IR, SEM, particle size, and zeta potential analysis verified successful PEG functionalization and drug conjugation without chemical incompatibility. The increase in particle size and positive shift in zeta potential indicated enhanced stability and surface modification. SEM imaging further confirmed uniform coating and stable drug attachment on the nanotube surface.

In in vivo antiepileptic evaluation using the Maximal Electroshock Seizure (MES) model, the PEG-MWCNT–Phenobarbitone formulation exhibited significant anticonvulsant activity, improving seizure protection without inducing toxicity or mortality.

Overall, the study demonstrates that PEG-functionalized MWCNTs serve as an efficient nanocarrier for sustained Phenobarbitone delivery, enhancing therapeutic efficacy, stability, and patient compliance. The optimized formulation shows strong potential for advanced antiepileptic therapy and can be further explored for targeted brain drug delivery applications.

8.REFERENCE

- Jawahar N, Surendra E, Krishna KR. A review on carbon nanotubes: A novel drug carrier for targeting to cancer cells.JPSR .2015;7(3):141.
- Kaur R, Vatta P, Kaur M. Carbon nanotubes: a review article. Int. J. Res. Appl. Sci. Eng. Technol. 2018 ;6(4):5075-7.
- Pitroda J, Jethwa B, Dave SK. A critical review on carbon nanotubes. Int. J. Constr. Res. Civ. Eng. 2016;2(5):36-42.
- Patel A, Prajapati P, Chaudhari K, Boghra R, Jadhav A. Carbon nanotubes production, characterisation and its applications. J. Adv. Pharm. Sci. 2010;1:187-95.
- Dey PR, Das NI. Carbon nanotubes: It's role in modern health care. Int. J. Pharm. Pharm. Sci. 2013;5(4):9-13.
- Gupta N, Gupta SM, Sharma SK. Carbon nanotubes: synthesis, properties and engineering applications. Carbon Letters. 2019 ;29(5):419-47.
- Hsieh CH, Jeng YL, Faiza M, Chun CH, Toyoko I. drug loading capacity and nuclear targeting of multiwalled carbon nanotubes grafted with anionic amphiphillic copolymers. Int. J. Nanomedicine. 2013;19(8):4427-40.
- Ul Quasim SZ, Ali MI, Irfan S, Naveed A. Advances in drug delivery system: carbon nanotubes.RJPT. 2013 ;6(2):75-79.
- Mishra V, Singh M, Nayak P, Sriram P, Suttee A. Carbon nanotubes as emerging nanocarriers in drug delivery: An overview. Int. J. Pharm. Qual. Assur. 2020;11:373-8.
- Sanket T, Shiv G, Pramod S, Ajay P. Carbon nanotubes in pharmaceutical nanotechnology: an introduction to future drug delivery system. Indo Am. J. Pharm. Res. 2013;3:3512-34.
- Kantamneni H, Gollakota A. Carbon nanotubes based systems for targeted drug delivery: a review. Int. J. Eng. Res. Technol. 2013 ;2:1-8.
- Khabashesku VN. Covalent functionalization of carbon nanotubes: synthesis, properties and applications of fluorinated derivatives. Russ Chem Rev. 2011;80(8):705-25
- Kumar R, Dhanawat M, Kumar S, N Singh B, K Pandit J, R Sinha V. Carbon nanotubes: a potential concept for drug delivery applications. Recent patents on drug delivery &

formulation. 2014;8(1):12-26.

- Kumar, V. et al., *Journal of Controlled Release*, 2023, "Functionalized Carbon Nanotubes as Nanocarriers for Central Nervous System Drug Delivery."
- Sharma, P. & Singh, R., *Neurochemistry International*, 2022, "Carbon Nanotube-Based Drug Delivery Approaches in the Management of Epilepsy."
- Al-Faraj, A. et al., *Nanomedicine: Nanotechnology, Biology and Medicine*, 2021, "Brain Targeted Delivery of Antiepileptic Drugs Using PEGylated Multi-Walled Carbon Nanotubes."
- Liu Z, Chen K, Davis C, Sherlock S, Cao Q, Chen X, et al. Drug delivery with carbon nanotubes for in vivo cancer treatment. *Cancer Res.* 2008;68(16):6652–60.
- Guo J, Hong H, Chen G, Shi S, Nayak TR, Theuer CP, et al. PEG-functionalized carbon nanotubes for imaging and drug delivery in cancer therapy. *Biomaterials.* 2013;34(23):5676–87.
- Antony J. *Design of Experiments for Engineers and Scientists.* 2nd ed. Oxford: Elsevier; 2014.
- Guo B, Liao C, Liu X, Yi J. Preliminary study on conjugation of formononetin with multiwalled carbon nanotubes for inducing apoptosis via ROS production in HeLa cells. Drug Des Devel Ther. 2018; 12:2815-26.
- Chudoba D, Jażdżewska M, Ludzik K, Wołoszczuk S, Juszyńska-Gałązka E, Kościński M. Description of release process of doxorubicin from modified carbon nanotubes. Int J Mol Sci. 2021; 22(21). 1-20.
- Kamazani FM, Siadat SD, Pornour M, Sheikhpour M. A success targeted nano delivery to lung cancer cells with multi-walled carbon nanotubes conjugated to bromocriptine. Sci Rep. 2021; 11(1):1–15.
- Khatti Z, Hashemianzadeh SM, Shafiei SA. A molecular study on drug delivery system based on carbon nanotube compared to silicon carbide nanotube for encapsulation of platinum-based anticancer drug. Adv Pharm Bull [Internet]. 2018; 8(1):163–7. Available from: https://doi.org/10.15171/apb.2018.020
- Wang C, Li W. Preparation, characterization, and in vitro and vivo antitumor activity of oridonin-conjugated multiwalled carbon nanotubes functionalized with carboxylic group. J Nanomater. 2016:1-8.
- Potraj B, Ahamed SM, Narasimha S, Suhas DS, Vijayakumar S. Deciphering the role of Bixin isolated from Bixa orellana L., in epileptic and psychotic experimental models in rodents. Man J Pharm Sci. 2019;4(2):15-22.
- Durg S, Veerapur VP, Thippeswamy BS, Ahamed SM. Antiepileptic and antipsychotic activities of standardized Śilājatu (Shilajit) in experimental animals. Anc. Sci. Life. 2015;35(2):110.