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Formulation and evaluation of carbon nanotubes for topical drug delivery

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***Abstract*---**The carbon nanotubes represent an innovative platform for controlled and targeted drug release in several biomedical applications. Carbon nanotube is one of the most efficient dispersed nano-systems with narrow size distribution ranges from 10-240 nm. Carbon nanotubes possess the remarkable electrical, thermal, mechanical conductivity which enables carbon nanotubes to be functionalized to enhance the solubility as well as biocompatibility of poorly bioavailable drugs. Further it can be converted to normal conventional system which is easy to use such as gel. The purpose of this study is to develop a stable nanotube based gel for topical application of fluconazole (BCS Class-II), having low solubility and high permeability to improve its solubility and hence cutaneous deposition to give local effect. The functionalization of pristine nanotubes was done using PEG-400 initially. Six batches (F1-F6) of drug loaded functionalized nanotubes was prepared using ethanol and water (2:1) which was tested for % drug loading and high drug loaded

batch was selected for conversion to gel (NTG1-NTG4) with carbopol 934 and HPMC as gelling agent. Formulations were evaluated for FTIR studies, % drug loading. From the result obtained, F6 formula shows better drug loading and considered as optimized, which is converted to gel and evaluated for appearance, pH, spreadability, viscosity, in vitro drug release study etc. Results of gel formulation are obtained as bluish black gel with pH 6-6.5, spreadability 42 – 68 gm.cm/sec, viscosity 42000-68500 cps & NTG3 shows highest 91.93% drug diffused while NTG1 shows lowest 81.37% drug diffused. Optimized formulation was subjected for stability studies. Nanotube gel have the potential applications in pharmaceutical industries because of high surface area, the higher rate of absorption; diffusion/ dissolution and which increased bioavailability of many conventional pharmaceuticals.

***Keywords*---**fluconazole, pristine nanotubes, PEG, drug loading, drug diffusion, stability study.

Introduction

Topical drug delivery can be defined as application of drug through skin to treat or cure the skin ailments. These systems are generally used for local skin infections like fungal infection. Topical preparations are used for the localized effects at the target site of their application by virtue of drug penetration into the underlying layers of skin or mucous membranes. Various topical formulations are to be administered like gel, cream, powders, solutions, and even medicated adhesive systems. The topical drug delivery system usually used for claim of drug holding preparation to the skin to directly treat cutaneous disorders or the cutaneous signs of a common ailment or disease with the intent of sequestering the pharmacological action or other effect of drug to the surface of the skin or within the skin. Topical activities may or may not require intracutaneous penetration or deposition. Topical delivery refers to the transfer of bioactive ingredients across the skin and into the systemic circulation. Dermatological products applied to skin is diverse in formulation and range in consistency from liquid to powder but the most popular products are semisolid preparation. Within the major group of semisolid preparations, the use of transparent gels has expanded both in cosmetics and in pharmaceutical preparations. These are superior in terms of use and patient acceptability. In unkindness of many advantages of gels, a major limitation is in the delivery of hydrophobic drugs. So to overcome this limitation, carbon nanotube gels are prepared and used so that even a hydrophobic therapeutic moiety can enjoy the unique properties of gels.

The choice of different novel drug delivery system has been used in which carbon nanotube plays an essential role in delivering the active pharmaceutical ingredient at the target organ or site. Carbon nanotubes (CNTs) are made up of graphite sheets rolled up into cylindrical tubes with a nanometre diameter, are relatively new allotrope since its first discovered in 1991 by Sumio Iijima a Japanese scientist. Carbon nanotubes (CNTs) possess remarkable electrical, mechanical, optical, thermal and chemical properties. In order to solve the problem of solubility and to improve the biocompatibility, the surface properties of

carbon nanotubes (CNTs) are modified by using functionalization techniques. The functionalization to carbon nanotubes will help an API to enclose within it and carry towards desired site without degrading by invivo environment of body. These properties make carbon nanotube unique providing positive advantage in new drug delivery system.

Materials and Methods

Fluconazole is received as gift sample from Yarrow Chem products. Pvt. Ltd., Carbon nanotubes purchased from Adnano Technologies Pvt. Ltd., Shimoga-577222, Karnataka, India, PEG 400, Oleic acid, Carbopol 934, HPMC, Glycerin, Methyl paraben, and Triethanolamine; all chemicals and solvents are of analytical grade A.

Functionalization of Pristine Nanotubes

500 mg of pristine Carbon nanotubes were dispersed in 100ml PEG solution (5gm/ml) with the aid of a fast clean ultrasonic bath. Sonicate for 15 mins. Removal of unbound PEG and agglomerated Carbon Nanotubes was done by centrifugation process at 7000 RPM for 5 Mins. Therefore, the supernatant was retained. Agglomerates and unbound PEG-400 was seperated by centrifugation and vaccum filtration followed by drying.

Preparation of Drug loaded nanotubes

A stock solution of fluconazole was prepared in ethanol and water (2:1), respectively, at a concentration of 1 mg/ml. The drug was loaded by incorporating the required weight of the functionalized nanotubes composite into the required volume of drug solution by sonication for 10 minutes and stirred for 16 hours at room temperature. The Drug loaded nanotubes were collected by centrifugation at 3000 rpm, on 4°C for 10 minutes by removing clear supernatant, sediment of drug loaded nanotubes carefully collected, dried and stored. These drug loaded nanotubes were evaluated for % drug loading and on that basis high drug loaded batch was selected for further studies.

Table 1: Drug loading into Functionalized CNTs

Batch	F1	F2	F3	F4	F5	F6
Drug(mg)	50	50	50	50	50	50
PEG-NT(mg)	50	60	70	80	90	100

Formulation of Nanotube Gel

Nanotube based gel was prepared as per table no. 2 by dispersing the weighed amount of the Carbopol-934 and HPMC in a sufficient quantity of distilled water. After complete dispersion, the solution was kept in dark for 24 hrs for complete swelling of carbopol-934. The Carbopol and HPMC dispersion was mixed with selected formulations containing 100 mg of drug loaded carbon nanotubes in excipients. The mixture was stirred well to get homogenous solution so that concentration of Carbopol 934 will become 0.5% w/v. If necessary

triethanolamine was added to maintain the pH with continuous stirring to get homogenous gel.

Table 2: Formulation of Nanotube Gel

Formula code	Drug loaded Nanotubes (mg)	Gelling agent % W/V		Water (ml)	Methyl Paraben (ml)	Glycerin (ml)
		Carbopol 934	HPMC			
NG1	100	1	1	50	0.1	5
NG2	100	1	1.5	50	0.1	5
NG3	100	1	2	50	0.1	5
NG4	100	1	2.5	50	0.1	5

Evaluation Parameters

Drug Characterization

Physical appearance

Fluconazole sample which was supplied from Yarrow Chem products. Pvt. Ltd was observed for physical appearance i.e. colour, odor, nature, etc.

Determination of Melting point

The drug was characterized for melting point. The melting point was resolve by filling small amount of fluconazole substance in the capillary tube then it attached to thermometer and continuous heat was applied with assembly suspended in the Thiel's tube including paraffin bath. The temperature at which the drug melts was noted as melting point.

Solubility study

For the purpose of solubility, additional amount of drug is added in the solvent in the (water, methanol, ethanol, chloroform, etc.) at room temperature and kept for 24 hrs with rare shaking. The supernatant was taken and evaluated by using shimadzu UV 1800 double beam spectrophotometer.

Estimation of Fluconazole by UV-spectroscopy method

Accurately weighed 50 mg of Fluconazole was dissolved in 50 ml of methanol in 50 ml volumetric flask to get the stock solution of 1000 µg/ml. From stock solution 5 ml was pipetted out and further diluted up to 50ml with buffer to get 100 µg/ml solutions. From 100µg/ml solution take 5ml and diluted to 50ml to get 10µg/ml solution from these aliquots of 2, 4, 6, 8, 10 ml were withdrawn and diluted to 10 ml with buffer to obtain a concentration range of 2-10µg/ml. The absorbance of the solutions was measured at 212 nm by using UV-spectrophotometer. A graph of Concentration vs. Absorbance was plotted.

Fourier transforms Infra-red Spectroscopy (FTIR)

The FTIR study was performed on drug as well as excipients and formulation to find out the compatibility in between them, over the range of 4000-400 cm^{-1} in FTIR spectrometer. The FTIR study performed using Perkin Elmer FTIR.

Nanotube

Organoleptic properties

The drug loaded nanotubes was evaluated for colour and appearance.

Microscopy

Pristine nanotubes, funtionalized nanotubes and drug loaded nanotubes were seen under light microscope for surface morphological study.

Drug entrapment efficiency (EF%) in Carbon Nanotubes

10mg of fluconazole loaded functionalized carbon nanotubes was mixed with 100 ml of phosphate buffer (pH7.4), followed by centrifugation at 10000 rpm for 1 hour. To remove the entrapped drug from nanotubes 1ml of supernatant solution is diluted as required and calculate the concentration of drug by using UV spectrophotometer at 210 nm. The percentage entrapment efficiency (EE %) was calculated using the following equation:

$$\text{Drug Entrapment Efficiency (EF\%)} = \frac{\text{Weight of loaded drug}}{\text{Weight of feeding drug}} \times 100$$

Nanotube Gel

Appearance

The formulations were observed for the presence of any particulate matter. Clarity is one of the most important features of topical preparations. The appearance and clarity is determined by visual testing.

pH

The pH of carbon nanotube gel formulation is determined by using digital pH meter.

Drug content

2 gm of carbon nanotube gel from each formulation were taken in 100 ml volumetric flask having 10ml methanol and stirred by magnetic stirrer for 5 minutes. The solutions were filtered by using whatmann filter paper. The absorbance of the solution was estimated by UV spectrophotometer (UV 1800, Shimadzu) at 210 nm using standard curve against blank.

Determination of viscosity

Viscosity of drug containing carbon nanotube gel was determined by using Brookfield viscometer. 20 gm of nanotube gel was filled in a 50 ml beaker and the viscosity was measured by using Spindle number 6 at 10 rpm.

Spreadability

It is determined by apparatus which consists of a wooden block, which is provided by a pulley at one end. The spreadability is measured on the basis of 'Slip' and 'Drag' characteristics of carbon nanotube containing gel. A ground glass slide is fixed on this block. An excess of nanotube gel (about 2 g) under study is placed on this ground slide. The nanotube gel is then sandwiched between this slide and another glass slide. A 1 g weight is placed on the top of the two slides for 5 minutes to expel air and to provide a uniform film of the nanotube gel between the slides. By putting weight of 1g, the time (in seconds) required by the top slide to cover a distance of 7.5 cm with the help of string attached to the hook is noted. A shorter interval indicates better spreadability, which is calculated by the formula:

$$S = \frac{ML}{T}$$

Where,
S=Spreadability,
M=Weight tied to upper slide,
L=Length of glass slides,
T=Time taken to separate the slides completely from each other.

In vitro drug diffusion study

The diffusion studies of the prepared nanotube gel were carried out in franz diffusion cell through a cellophane membrane. Gel sample (0.5gm) was taken in cellophane membrane and the diffusion studies were carried out at 37 ± 1 C° using 250 ml of (25%) methanolic phosphate buffer (pH 7.4) as the dissolution medium. 5ml of each sample was withdrawn periodically at 1, 2, 3, 4, 5, 6, 7 and 8 hrs and each sample was replaced with equal volume of fresh dissolution medium in order to maintain sink condition. Samples were analyzed by UV-visible spectrophotometer at 210nm for drug diffused.

Stability study

Formulations of drug loaded nanotube gel are filled in glass container with proper aluminium foiling and placed for a short term accelerated stability study for 3 months at 40±2°C temperature and 75±5% relative humidity as per ICH Guidelines. After particular period of interval, the formulation was withdrawn and was evaluated for appearance, pH, drug content and in vitro drug release.

Results and Discussions

Drug Characterization

Drug was observed for organolleptic characteristics such as color, odor and nature; it complies specifications of IP tabulated in table no. 3.

Table 3: Organoleptic Characteristics

Properties	Specification as per IP 2010	Result
Colour	White	White
Odor	Odorless	Odorless
Nature	Amorphous	Amorphous

Melting point of drug was found to be in a range of 136-141 °C. Solubility of Fluconazole in various solvents was found as in table no. 4.

Table 4: Solubility Study

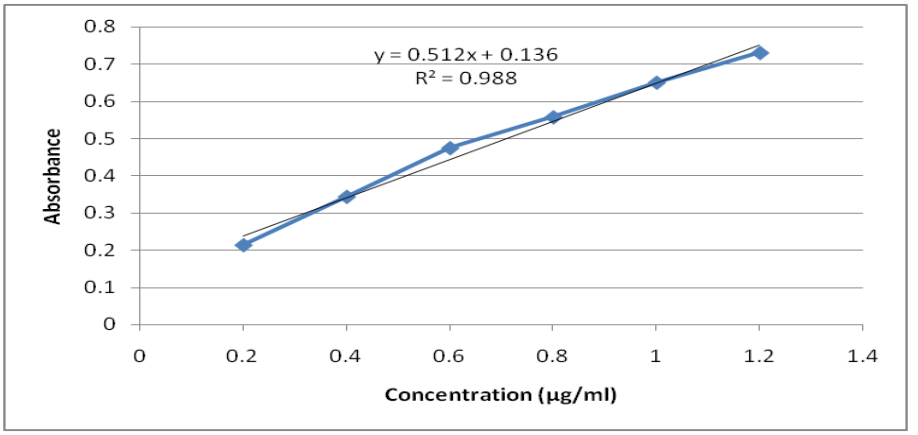
Sr. No.	Solvent system	Result
1	Ethanol	Freely soluble
2	Methanol	Freely Soluble
3	Water	Slightly soluble
4	Chloroform	Insoluble
5	Ether	Insoluble

The UV absorbance's of fluconazole standard solutions in the range of 0.2-1.2 µg/ml of drug in buffer pH 7.4 showed linearity at λ max 210nm. The linearity was plotted for absorbance (A) against concentration (C) with R^2 value 0.989 and with the slope equation y = 0.544x + 0.013. The absorbance values and standard curve were in below table 5 and figure 1.

Table 5: Standard calibration curve of Fluconazole

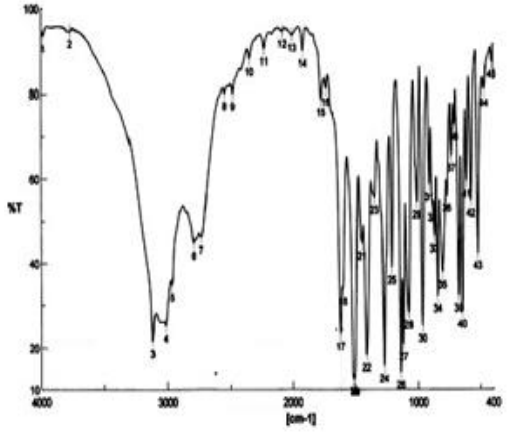
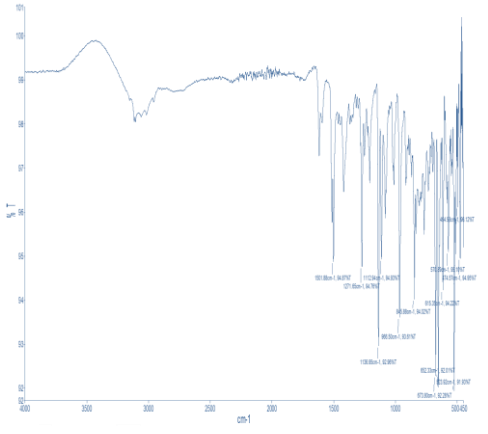
Concentration (µg/ml)	Absorbance
0.2	0.214
0.4	0.344
0.6	0.475
0.8	0.558
1	0.651
1.2	0.731

Figure 1: UV absorbance plot



The IR spectrum of the pure drug sample recorded by FTIR spectrometer was compared with standard peaks of Fluconazole as specified in IP. Results showed that frequencies of functional group of Fluconazole were in the reported range which indicates that the obtained sample was of Fluconazole and was pure. Results of FTIR peaks of drug were tabulated in table 6 as below.

Table 6: FTIR peaks of drug

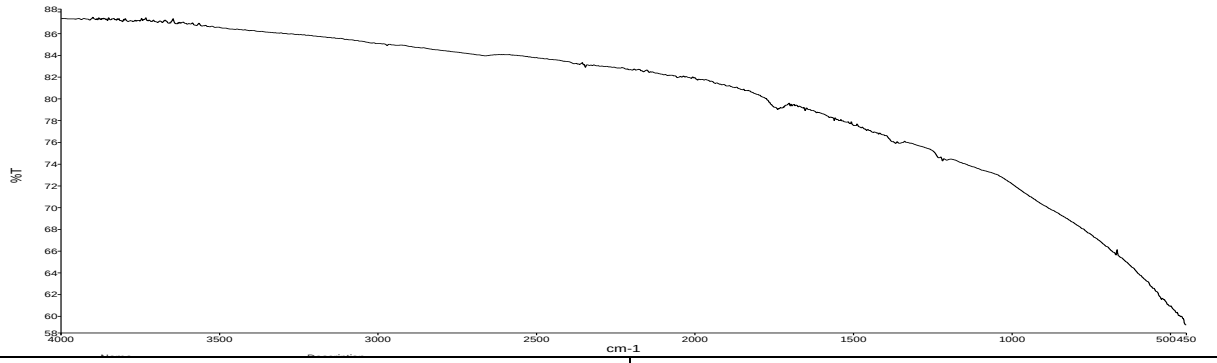
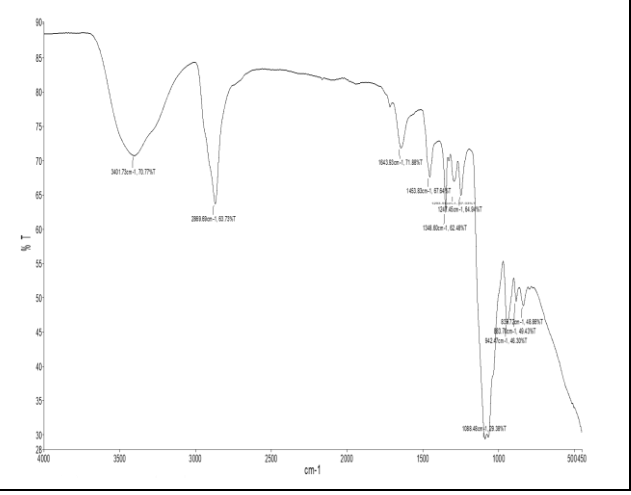
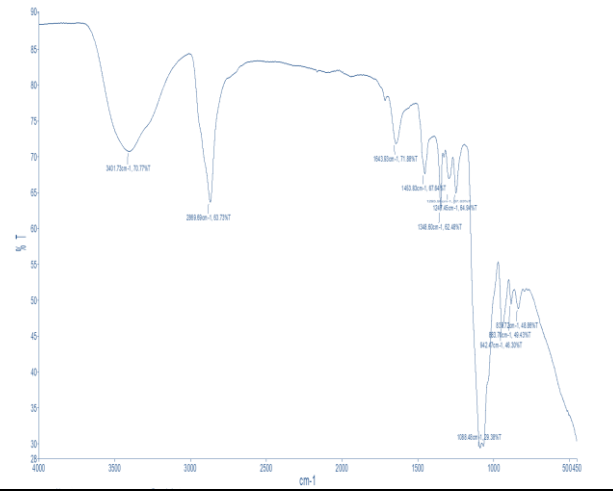
FTIR Peaks obtained for Std. Fluconazole	FTIR Peaks of Fluconazole supplied by Yarrow chem. Products pvt. Ltd.
	

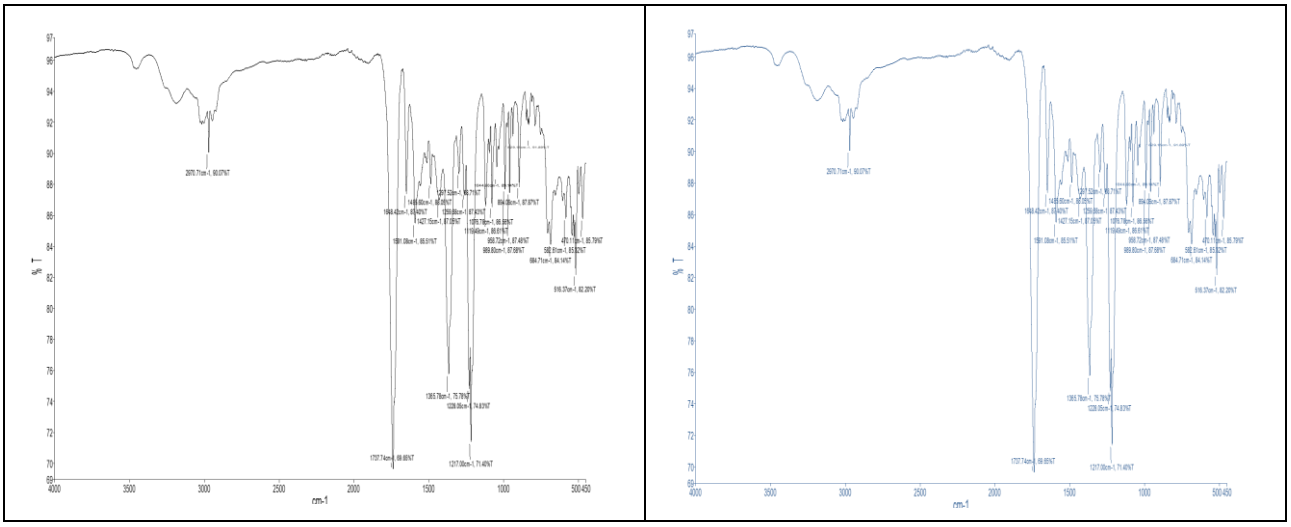
Evaluation of Nanotube

FTIR spectrum of plain NT does not show any peaks in comparison with functionalized NT which showed 11 peaks that confirmed functionalization of plain NT was successfully done. FTIR spectra of Functionalized nanotubes and drug loaded nanotubes, was obtained twice by taking interval of 1 month in between two testing; the results of two tests were confirmed that there were no

significant change and hence it can be concluded that the drug and excipients are compatible.

Table 7: FTIR peaks of Pristine Nanotubes, Functionalized nanotubes, Drug loaded nanotubes

Pristine Nanotubes	
	
FTIR Peaks obtained initially	FTIR Peaks obtained after 1 month
Functionalized nanotubes	
	
Drug loaded nanotubes	

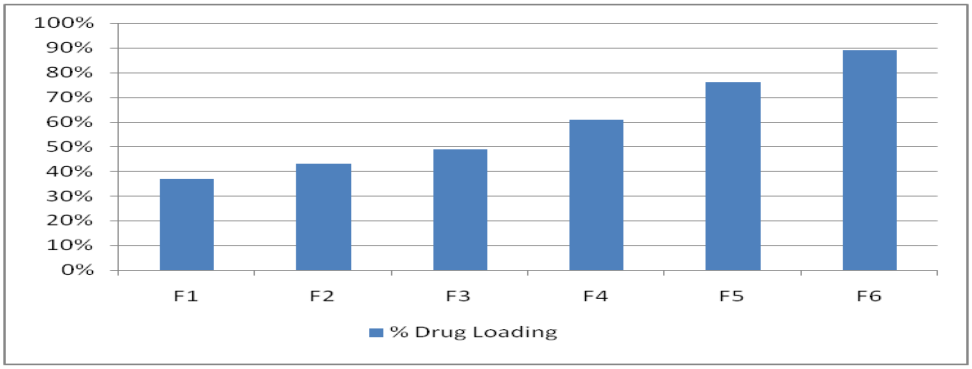


The nanotube formulation was bluish-black, in colour. The % drug loading of all formulation was found to be 37-89% . Among all the formulation F6 shows highest drug loading efficiency. The drug content of F6 was found to 89%. The results are shown in the table no.8.

Table no.8: % drug loading into the PEG-NT

Batch	F1	F2	F3	F4	F5	F6
% Drug Loading	37%	43%	49%	61%	76%	89%

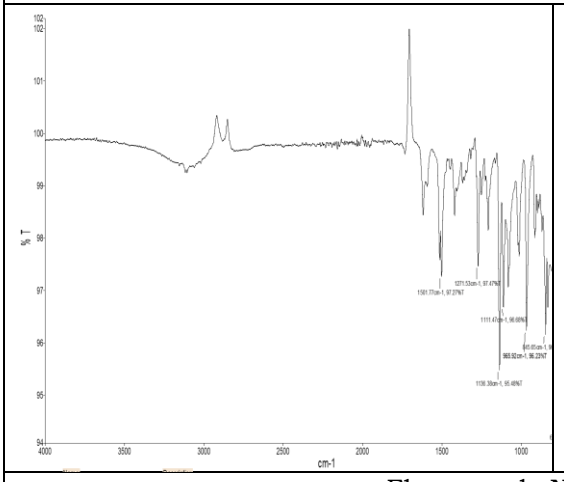
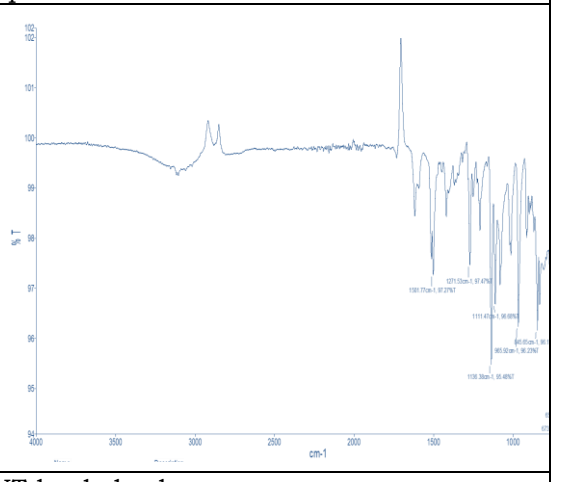
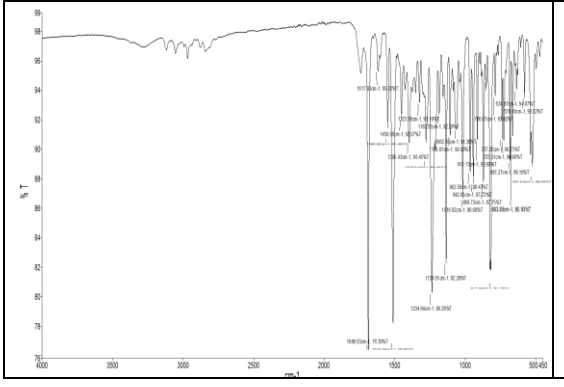
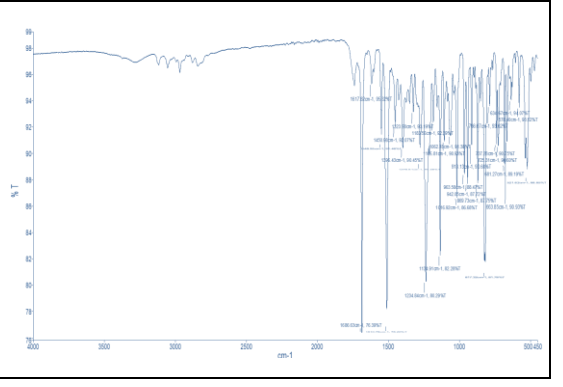
Figure no.2 % Drug loading into PEG-NT



Evaluation of Nanotube gel

FTIR evaluations of Fluconazole NT+Carbopol+HPMC+Oleic acid, and Fluconazole NT loaded gel was obtained twice by taking interval of 1 month in between two testing; the results of two tests were confirmed that there were no significant change and hence it can be concluded that the drug and excipients are compatible.

Table no.9: FTIR evaluations of Fluconazole NT +Carbapol+HPMC+Oleic acid, and Fluconazole NT loaded gel

FTIR Peaks obtained initially	FTIR Peaks obtained after 1 month
Fluconazole NT +Carbapol+HPMC+Oleic acid	
	
Fluconazole NT loaded gel	
	

The nanotube gel was bluish-black in colour, homogeneous in nature and pH of nanotube gel was found to be in between 6 - 6.5, which lies in between normal pH range of skin which may not produce skin irritation. The nanotube gel was analyzed for drug content spectrophotometrically at 210 nm. The drug content was found to be in the range of 78 to 90%. Drug content of NTG3 is highest i.e. 90.23% and hence it was considered as best one.

Depends on the concentration of gelling agent nanotube gel has viscosity ranging from 42000-68500 cps and spreadability from 42 – 68 gm.cm/sec.

Table 10: Evaluation results of nanotube gel

Formulation code	Appearance	pH	% Drug content	Viscosity (Cps)	Spreadability (gm.cm/sec)
NTG1	Transparent Bluish-black gel	6.5	78.88	42700	54.52
NTG2	Transparent	6.4	87.90	46800	42.85

	Bluish-black gel				
NTG3	Transparent Bluish-black gel	6.5	90.23	52400	67.18
NTG4	Transparent Bluish-black gel	6.3	80.45	68400	60.46

Figure 3: % Drug content studies

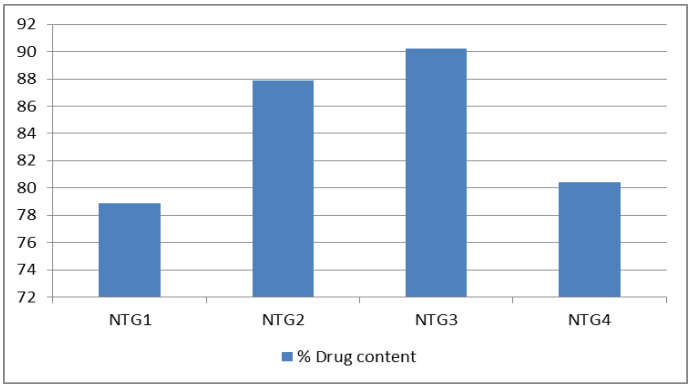


Figure 4: Viscosity (Cps) studies

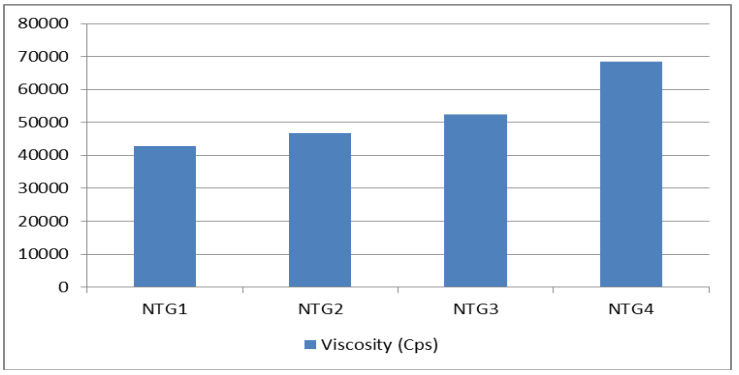
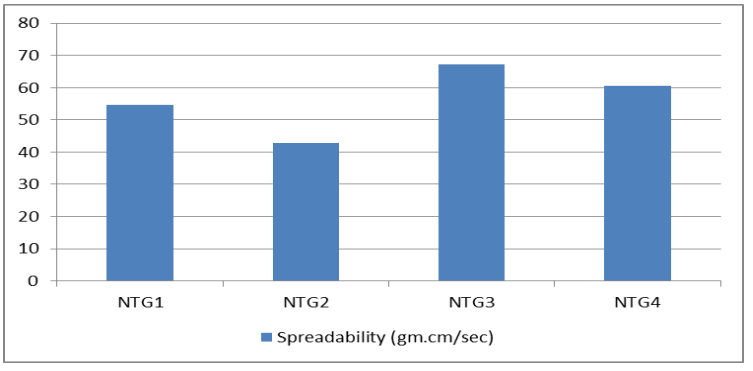


Figure 5: Spreadability studies

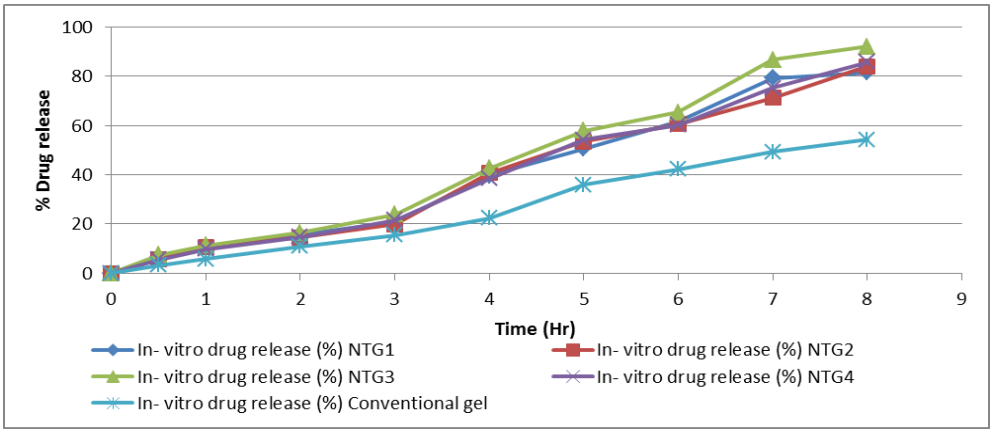


The in vitro diffusion of Fluconazole from the nanotube gel was varied in amount according to concentration of PEG-CNT used in formulation. Comparative in- vitro drug release study of gel are tabulated in table no. 11. From the study it was concluded that nanotube gel NTG3 showed highest diffusion of Fluconazole within 8 hr. which is optimized batch, further subjected to stability study.

Table 11: Comparative in- vitro drug release study of gel

Time in (hr)	In- vitro drug release (%)				
	NTG1	NTG2	NTG3	NTG4	Conventional gel
0	0	0	0	0	0
0.5	6.54	5.82	7.43	5.66	3.32
1	10.83	10.70	11.32	9.79	5.85
2	15.68	14.50	16.54	14.77	10.86
3	20.89	19.86	23.86	21.60	15.56
4	39.75	40.69	42.60	38.43	22.37
5	50.51	53.39	57.73	54.12	35.84
6	61.72	60.55	65.23	60.05	42.21
7	79.25	71.15	86.57	75.17	49.20
8	81.37	83.95	91.93	85.63	54.23

Figure 6: % In vitro drug release



The accelerated stability study of nanotube gel NTG3 was carried out; there is no significant change in pH, drug content and % drug release in NTG3 formulation at 40℃ temperature and 75% RH. Results of stability study are given in table no.12, 13and figure 5.

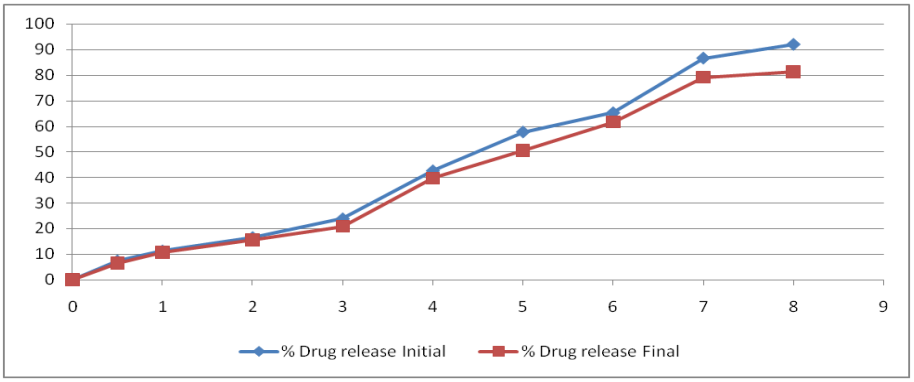
Table 12: Stability study of optimized nanotube gel

Formulation code	Month 1		Month 2		Month 3	
	pH	%Drug content	pH	%Drug content	pH	%Drug content
NTG3	6.5	90.23	6.5	89.73	6.5	88.94

Table 13: % Drug release after accelerated stability study of optimized formulation (NTG3)

Times (hrs)	% Drug release	
	Initial	Final
0	0	0
0.5	7.43	5.74
1	11.32	10.43
2	16.54	14.68
3	23.86	21.67
4	42.60	39.31
5	57.73	52.43
6	65.23	61.22
7	86.57	78.15
8	91.93	81.57

Figure 7: Accelerated stability study



Conclusion

The research conducted has successfully encapsulated poorly bioavailable drug fluconazole into functionalized carbon nanotubes with high drug loading ability (89%) which is further formulated as topical gel. Result has showed maximum drug release within 8 hrs (91.93%) with good drug content (90.23%) which can be achievable with nanotube's topical gel. Additionally nanotubes require only small amount of drug to get therapeutic action, hence it does not give any side effect, by directing drug to specific site. Although if nanotube's has better results; no one can predict its efficacy hence clinical studies are needed to understand its biological metabolism.

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