FORMULATION AND EVALUATION OF CHITOSAN NANOSPHERES

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ABSTRACT

The word "nanotechnology," coined by Norio Taniguchi in 1974 at the University of Tokyo, offers chances to integrate nanoscale science and development in the physical sciences. The use of science and technology to the creation of items including or using tiny particles is known as nanotechnology. As a result, it is used in many fields of biomedical sciences. The development of biomarkers, this technique enables cellular treatment, medication distribution, tissue regeneration, cell membrane smuggling, bio imaging, and gene editing.

Keywords: Nanotechnology, Nanospheres, Chitosan
DOI Number: 10.48047/NQ.2022.20.16.NQ880302 **NeuroQuantology2022;20(16): 2957-2974**

Nanotechnology is defined by the US Food and Drug Administration as "technology and research at the nuclear, biochemical, or molecular stage, in the size scale of about 1-100 nm; developing and using structures, devices, and devices with new properties and performance due to their small and/or intermediate size; and the able to handle or manipulate on an atomic scale." In the near future, nanospheres get a wide range of potential applications in novel skincare products, fabrics, and paints. These technologies have the potential to treat previously incurable diseases as well as enhance the effectiveness of traditional novel molecular treatments (Shinde et al., 2012)

The advancement of nanotechnology over the last two decades has had a significant impact on clinical therapeutics. Drug delivery has become more efficient and safer to hydrogel nanosized drug delivery carriers like lipid nanoparticles and niosomes. Nanoparticle drug delivery has several advantages, including longer exchange half-lives, better pharmacokinetic properties, and minimal side effects, notably at the systemic level. In cancer treatments, nanosponges can also rely on the elevated permeation and retainment effect is caused by clogged cancer microvessels for good drug buildup at tumour sites. Restorative Nanotubes have emerged as an intriguing alternative to classical treatment for cancer, in which therapeutic efficacy of toxic agents poses a

serious risk to soft cells and findings in amount of the drug side effects. Presently, several nanoparticle-based

Nanospheres are classified into two types:
1) Nanospheres are matrix-like systems in which substances are diffused in a polymer matrices.
2) Nanocapsule - The active ingredient in this drug is encased in a central quantity bounded by a constant polysiloxane layer (Nagavarma et al., 2012)

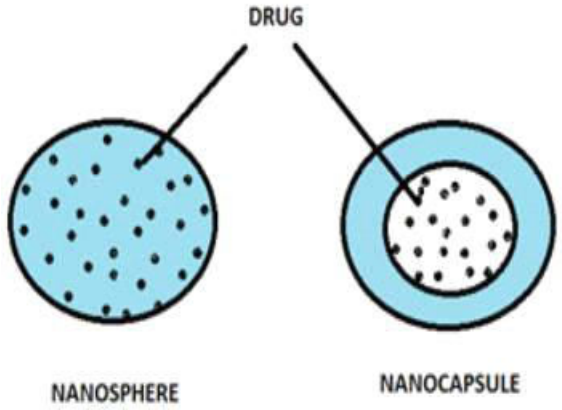

Figure: 1 Various type of Nanospheres

MATERIALS AND EQUIPMENTS

Table 1: List of instruments used and their manufacturer

NAME OF THE EQUIPMENT	MANUFACTURE
Weighing balance (AW 120)	Shimadzu , japan
UV-Visible spectrophotometer (Analytical , S L -210),	ElicopvtLtd. Ahmedabad India,
Magnetic stirrer	Remiequipments , Mumbai
Dissolution Apparatus(TDT-08L)	Electrolab , India
FT-IR	Perkin Elmer , U.S.A
Scanning electron microscopy(S-4100)	Perkin Elmer , U.S.A
Differential scanning calorimeter(DSC)	Metteler Toledo, Switzerland
Probe sonicator	Sonics, Germany
Particle size analizer	Shimadzu sald 2300

Preformulation Studies

The term preformulation defined as the study which is undertaken just before to articulating an active ingredient in work to explain the rate of the drug and its interplay with active ingredients.

Preformulation testing is the initial step in the systematic advancement of drug dosage forms. It is defined as the research of a drug substance's physical and chemical properties, alike away from the bustling and so when combined with excipients. The overarching objective of preformulation test

results is to generate data that the formulator can use to create secure and biologically available dosage forms.

Preformulation Analysis of Solubility Absorption screening was done in order to identify an useful suitable solvent for solubilizing the drug together with the numerous inert ingredients used in composition, along with to decide the drug's solubility.

Infrared spectroscopy:

Viltolarsen's IR absorption spectrum was determined using an FTIR spectrophotometer and the KBr dispersion method. The obtained infrared spectrum of the Nanoparticles was compared with the standard ir spectrum of the pure drug. FTIR spectra were employed to confirm the authenticity of the drug and identify the conversation of the drug with polymers in order to verify the suitability of the polymer and the drug.

To make 1% sodium laureth sulfate solution: 10 gm sodium lauryl sulphate was accurately weighed and added to 1,000 ml of distilled water.

Creating a standard graph in 1%SLS solution: 10 mg of Viltolarsen was dispersed in 1000ml of water that contains 10 gm of sodium laureth sulfate solution in a 1000ml conical flask. 5ml, 10ml, 15ml, 20ml, 25ml, and 30ml were taken.

Method of preparation:

Polymer and drug solution preparation:

1. Weighed and poured the necessary amount of polymer into a dry beaker.
2. In a measuring cylinder, the requisite quantity of solvent (methanol) was measured.
3. Methanol has been gradually added to the beaker containing the polymer.
4. A polymer solution was formed by continuously stirring it with a glass rod.
5. Add 300milligrammes Viltolarsen, weighed accurately, and thoroughly mix.

Viltolarsen As an advanced tool in the preparation of nanodrugs, nanospheres were prepared using a micelle preceded by solvent evaporation technique. Polyesters have been dissolved in chloroform and after which 10mg of Viltolarsen drug was totally distributed in polymer solution, and 1% SLS solution was then added to this while stir the mixture at 400-500 rpm for 20 minutes, before being placed in a probe sonicate beaker.

Formulation:

	LF1	LF2	LF3	LF4	LF5	LF6	LF7	LF8	LF9
Viltolarsen (mg)	300	300	300	300	300	300	300	300	300
HPMC K4M (mg)	75	150	225	-	-	-	75	150	225
Glycerylmonostearate(mg)	-	-	-	75	150	225	75	150	225
Ethyl cellulose (mg)	75	150	225	75	150	225	-	-	-
Dichloromethane (ml)	10	10	10	10	10	10	10	10	10
Methanol (ml)	10	10	10	10	10	10	10	10	10

2% SLS (ml)	50	50	50	50	50	50	50	50	50

Characterization of Nanospheres

Assay

Titrate with 0.1 mol/L sodium hydroxide VS after weighing 0.3 g of Viltolarsen (fabricated nano crystals) in 40 mL of methanol (potentiometric titration, Endpoint Detection Method in Titrimetry).
35.419 mg C16H13Cl2NO4 per ml of solution of 0.1 mol/L sodium hydroxide
Viltolarsen includes not less than 99.0% but not more than 101.0% Viltolarsen when dried.

Modified Dissolution Test:

Titrate with 0.1 mol/L sodium hydroxide VS after weighing 0.3 g of Viltolarsen (fabricated nano crystals) in 40 mL of methanol (potentiometric titration, Endpoint Detection Method in Titrimetry).
35.419 mg C16H13Cl2NO4 per ml of solution of 0.1 mol/L sodium hydroxide
Viltolarsen includes not less than 99.0% but not more than 101.0% Viltolarsen when dried.

FT-IR Spectroscopy:

IR spectral perfectly matched research are employed to detect potential drug-polymer or excipient interactions. The current study used FT-IR to assess the drug Viltolarsen's compatibility with various polymers (PERKIN ELMER FT-I Insf. USA). An FT-IR spectrophotometer was used to scan the samples from 4000 to 400 cm -1. Similarly, the infrared spectra of each drug and nanopowder were captured. The visual characteristics of the samples, as well as the presence or dissolution of peaks in the spectra, were observed to investigate any possibility physiochemical interactions.

Scanning Electron Microscopy (SEM):

The particulate shape both the unrefined drug as well as the fictitious drug Nanospheres was studied using SEM. A tiny part of each drug powder sample was faltered with a Pt-Pd alloy at a diameter of 5 nm on with the double conductive carbon tape. The photographs were taken with a Zeiss DSM 982 Field Emission Gun Scanning Electron Microscope (Carl Zeiss AG, Germany)**.**

Particle size distribution:

The shape of drug Nanospheres was determined immediately after precipitation using dynamic laser light scattering (Nanoparticle size analyzer, Malvern). Prior to analysis, the substance samples were diluted to 0.2 mg/ml in filtered water. Particle size analysis results were interpreted utilising visual mean size (Mz) and premeditated surface area (Cs).

Differential scanning calorimetry (DSC) measurement:

The thermophysical conductivity of lyophilized nanoparticle samples were investigated using a DSC-41 apparatus (Shimadzu, Japan). Each lyophilized particle sample had its scanning temperature set among 25 and 200 ° C., with such a heating rate of 10 ° C. per minute. Each sample was tested in an expansive aluminium pan with 10 mg of magnesia as a control. Thermal analysis of Viltolarsen and the absorbent was performed to evaluate the micro - structural changes after the nanosizing method.

Zeta Potential

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The nanospheres' size, size and shape, and zeta potential were determined using a zeta sizer. Before being analysed, the lyophilized samples were dilute with PBS to a concentration of 67 mm and a pH of 6.0 on mg/ml. These samples were first located in this other smooth cubet before even being located in the zeta size analysis compartment to collect different peaks and then the average zeta size. To obtain zeta potential data, surface charge possible or zeta potential samples were put in the zeta sizer assessment chamber and the high point was observed. During the assessment of these data, narrow size distribution nature is always preferred over polydisperse nature.

RESULTS AND DISCUSSION

Preformulation studies

The active drug ingredients were characterised.
In preformulation investigations, API was characterised (appearance, identification test by FTIR, assay), and it was discovered that all fall within the guidelines set out in the pharmacopoeia.

Table 3: Characterization of active pharmaceutical ingredient

Description	Specifications	Observations
Appearance	White Crystalline powder	White
Identification	FTIR	Complies
Assay	Not less than 99.0% w/w and not more than 101.0% w/w of Viltolarsen	99.97%w/w

Viltolarsen's standard graph in 1% SLS solution

The standard graph by Viltolarsen has been generated with 1% SLS. Concentrations of 2 to 10 g/ml were created. After calibrating with a blank sample, the absorption spectrum of the created concentrations was measured at 275 nm. A graph was created to depict concentration and absorbance, and the best fit line, correlation value, and formula were created to describe the data.

Table 4:

Concentration (µg/ml)	absorbance
0	0
4	0.2

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8	0.39
12	0.55
16	0.72
20	0.89

Figure 8: absorbance

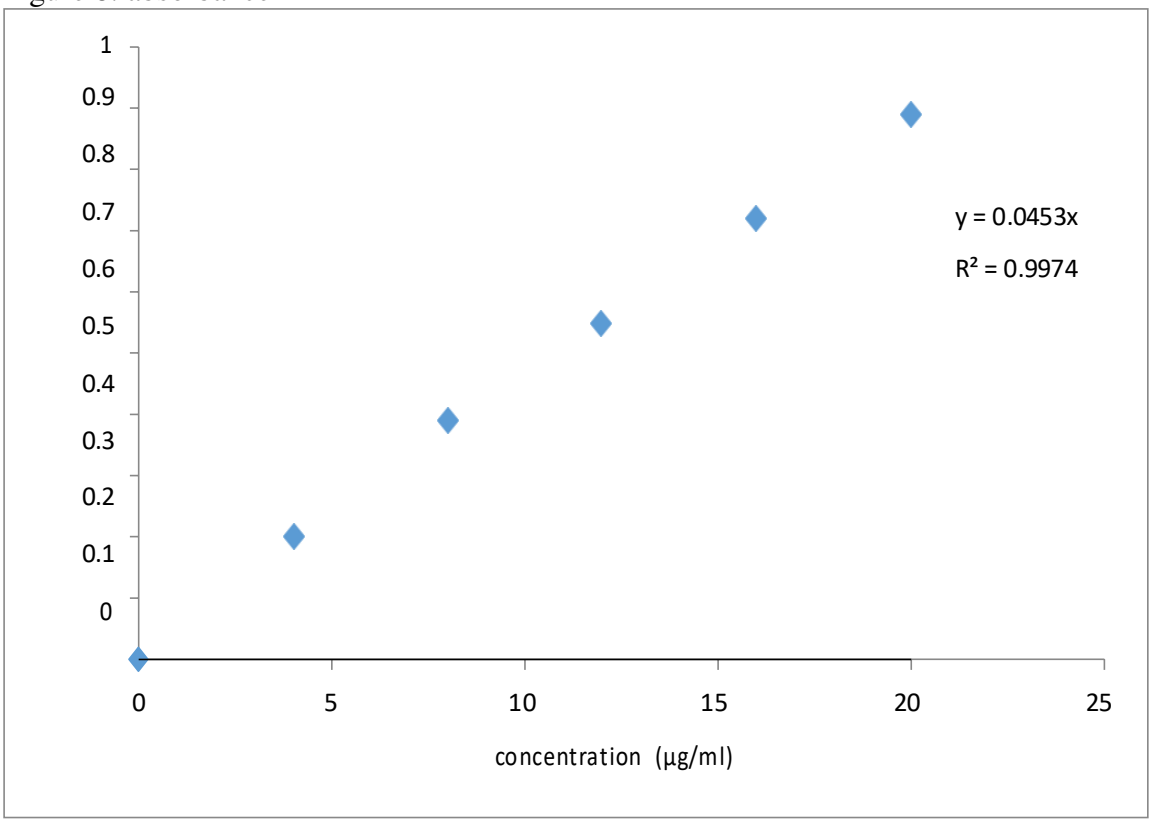

Table 5:

Evaluation of Nanospheres:

Formulation code	Particle size (nm)	% yield	Entrapment efficiency (%)	Drug content(mg)
F1	200.5	98.5	77.8	298.5

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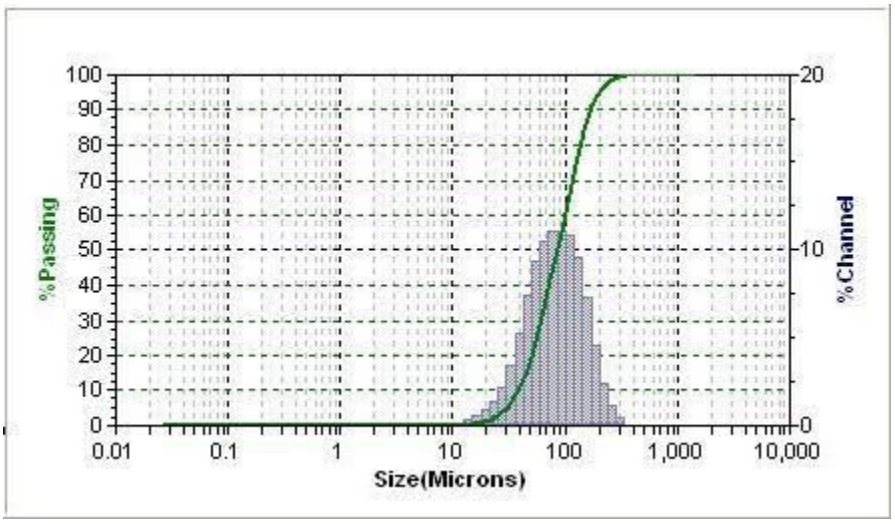
F2	210.2	80.7	87.5	297.8
F3	246.7	79.5	97.6	298.2
F4	198.2	96.2	75.2	298.0
F5	205.3	87.5	80.2	298.2
F6	226.7	79.8	91.8	297.4
F7	197.2	98.8	77.4	298.4
F8	220.2	84.2	83.4	296.3
F9	245.3	75.8	95.2	295.5



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F4	198.2	96.2	75.2	298.0
F5	205.3	87.5	80.2	298.2
F6	226.7	79.8	91.8	297.4
F7	197.2	98.8	77.4	298.4
F8	220.2	84.2	83.4	296.3
F9	245.3	75.8	95.2	295.5

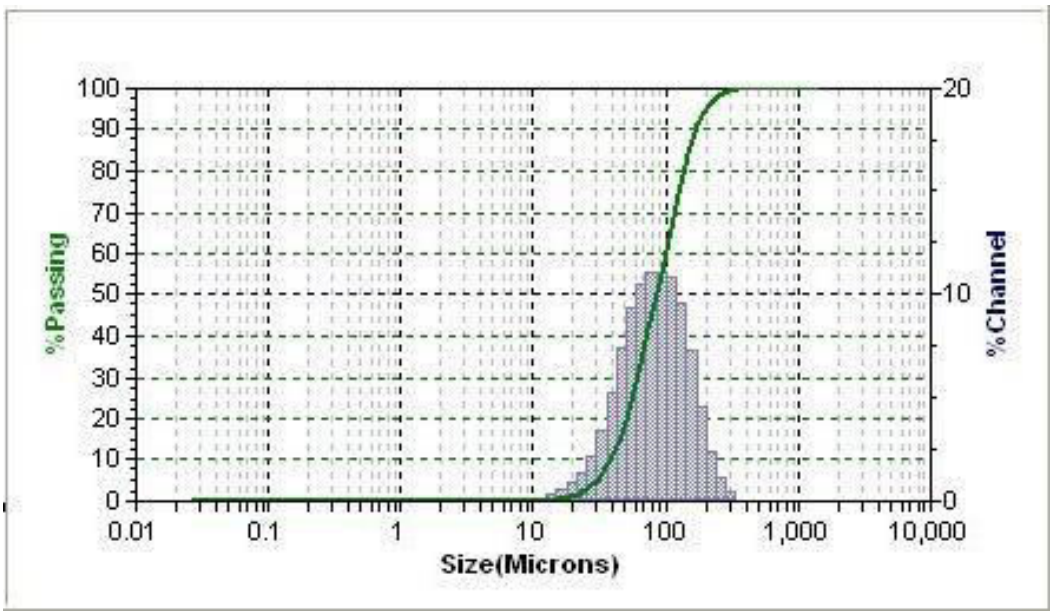
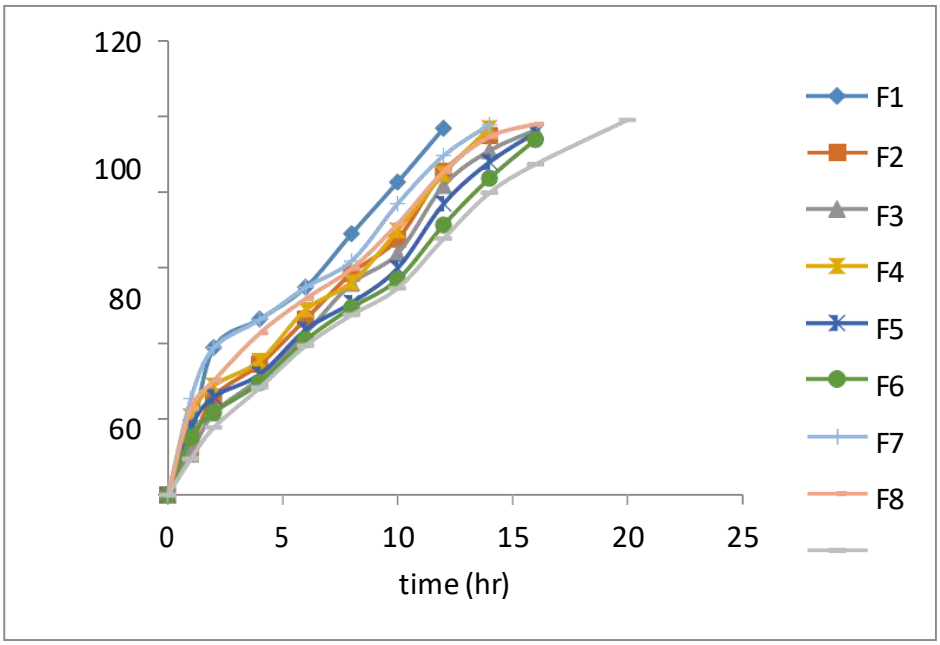


Table 6:

In vitro dissolution study:

Ti m e (hr)	% drug release								
	Formulation code								
	LF1	LF2	LF3	LF4	LF5	LF6	LF9	LF8	LF9
1	15.2± 0.21	12.5± 0.31	10.8± 0.42	20.8± 0.12	17.8± 0.33	15.2± 0.26	25.4± 0.23	22.8± 0.16	9.5±0. 41

2	38.9± 0.53	25.9± 0.29	21.6± 0.23	28.9± 0.54	25.9± 0.38	21.8± 0.19	38.9± 0.61	30.2± 0.43	17.8± 0.32
4	46.4± 0.41	34.5± 0.22	30.8± 0.22	35.4± 0.31	31.8± 0.44	29.6± 0.43	46.2± 0.41	42.7± 0.25	28.3± 0.79
6	54.8± 0.11	46.4± 0.64	42.7± 0.41	48.9± 0.16	43.6± 0.79	40.9± 0.36	54.8±	51.8± 0.38	39.4± 0.61
8	68.9± 0.78	58.5± 0.55	55.8± 0.31	56.1± 0.13	50.7± 0.25	49.4± 0.35	61.7± 0.18	59.7± 0.44	47.5± 0.45
10	82.5± 0.39	67.5± 0.53	63.7± 0.13	69.8± 0.34	59.8± 0.43	56.8± 0.42	76.8± 0.50	71.5± 0.60	54.5± 0.53
12	96.7± 0.14	85.4± 0.26	81.6± 0.52	84.7± 0.25	76.8± 0.15	71.2± 0.67	89.5± 0.28	85.3± 0.54	67.6± 0.61
14	-	94.8± 0.71	90.8± 0.63	96.8± 0.11	87.8± 0.09	83.5± 0.17	97.6± 0.17	94.5± 0.46	79.7± 0.28
16	-	-	96.2± 0.71	-	95.5± 0.43	93.7± 0.24	-	97.8± 0.45	87.2± 0.36
20	-	-	-	-	-	-	-	-	98.9± 0.76



FTIR Spectra For Viltolarsen

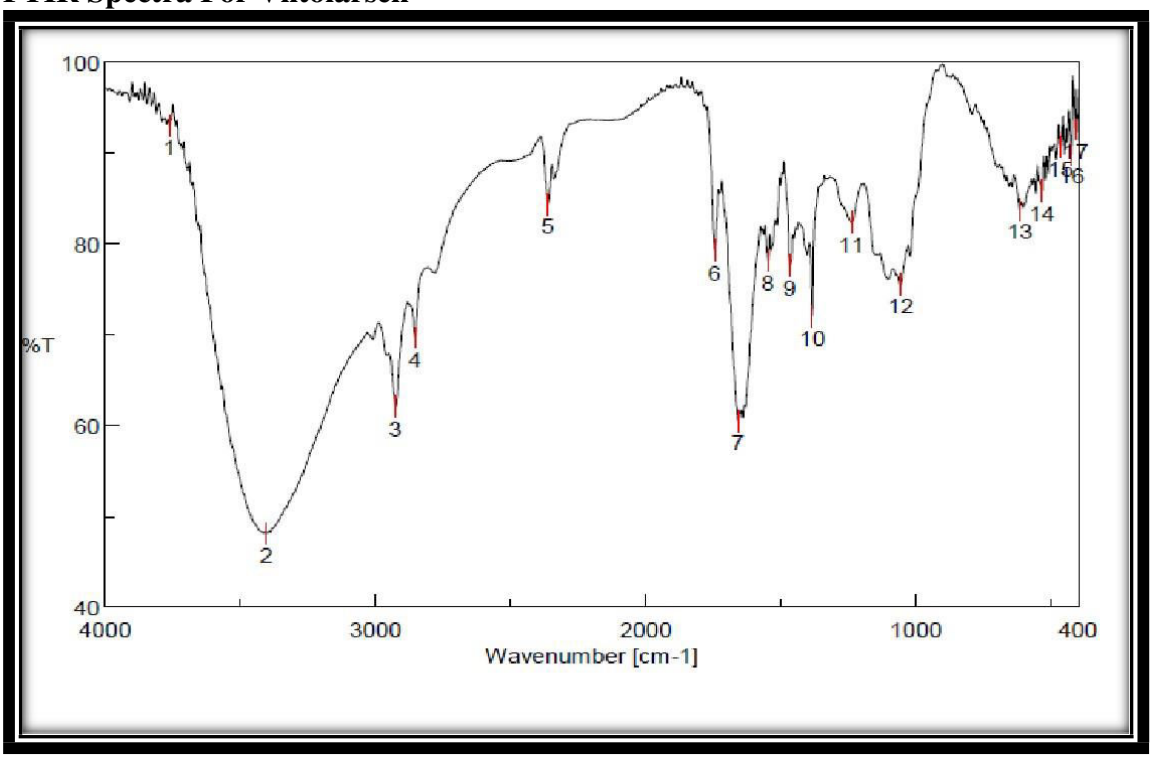


Figure:12 FTIR spectrum of Viltolarsen

Materials	Standard wave Number (cm-1)	Test wave Number (cm-1)	Functional group assignment
Viltolarsen	3000-3700	3404.71	OH stretching
	1500-1700	1547.59	NH bending
	1300-1500	1465.63	CH bending
	600-900	616.145	CH rocking
	1600-1900	1657.52	C-O stretching

Figure:13 FTIR spectrum of DRUG+Polymers

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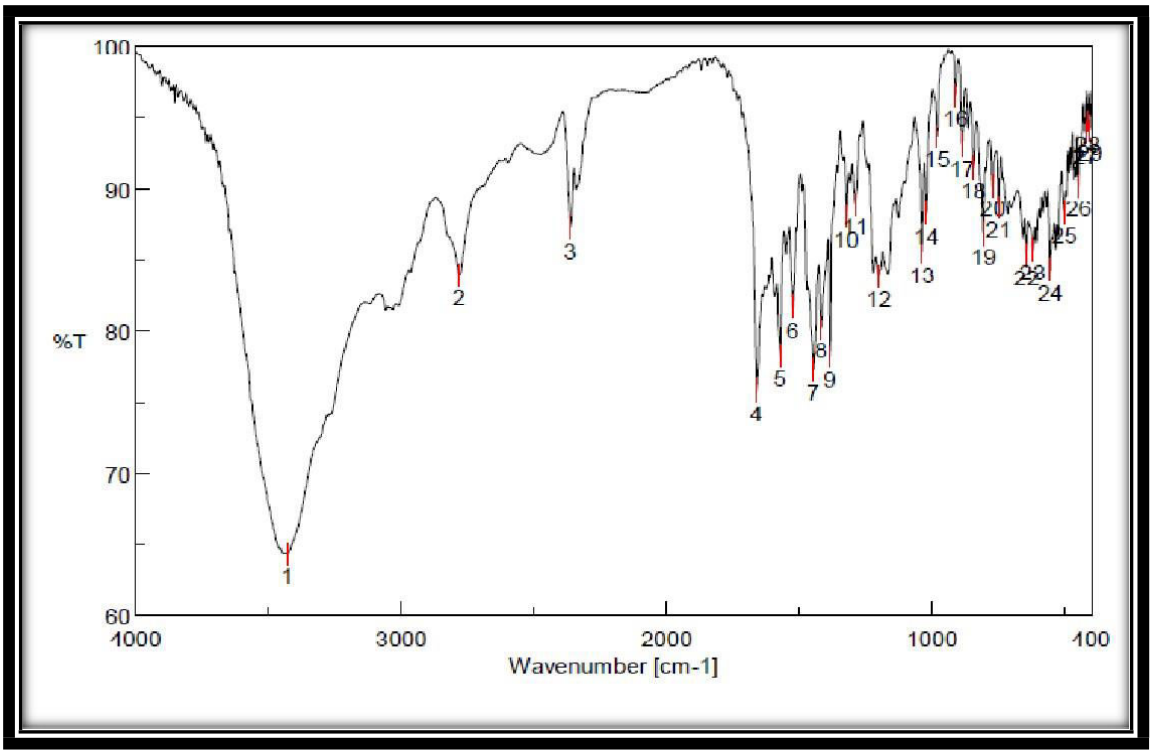

Materials	Standard wave Number (cm-1)	Test wave Number (cm-1)	Functional group assignment
DRUG+Polymers	3000-3700	3427.85	OH stretching
	1500-1700	1571.7	NH bending
	1300-1500	1384.64	CH bending
	600-900	843.704	CH rocking
	1600-1700	1662.41	C-C stretching

SCANNING ELECTRON MICROSCOPE ANALYSIS

Using a transmission electron microscope, the morphology of the Viltolarsen Nanospheres was clearly defined by globular features (SEM). The surfaces of the particles are rough and rounded. As according reports, the polymer ratio increased along with the predisposition for the relative diameters of the pores to increase.

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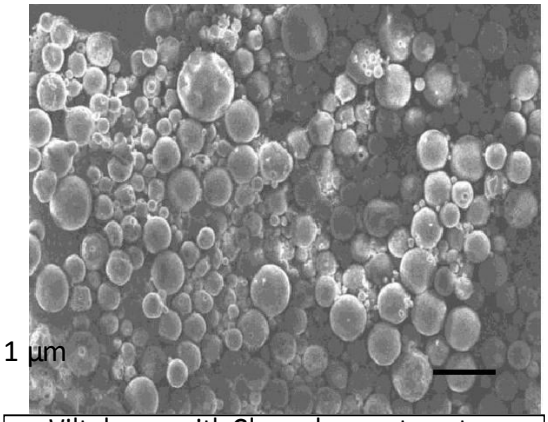
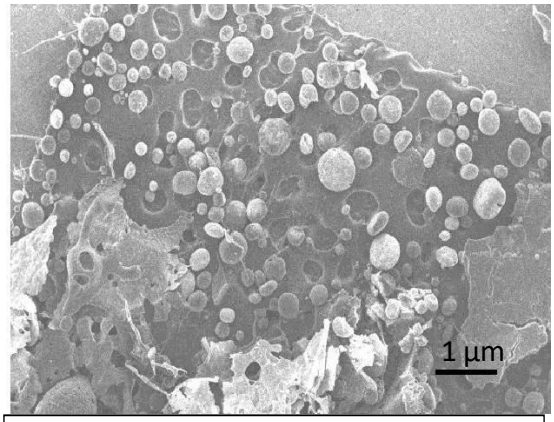


Viltolarsen with Glycerylmonostearate (80mg)



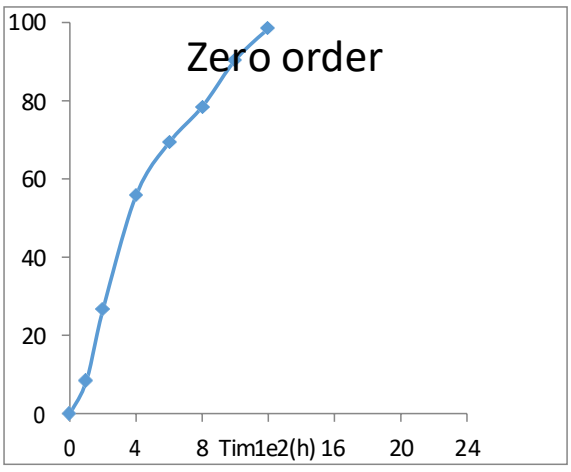
Viltolarsen with Glycerylmonostearate (100mg)

KINETIC ANALYSIS OF DISSOLUTION DATA:

The in-vitro release data was fitted into different release equations and kinetic models, including zero order, first order, Higuchi, and Korsmeyer Peppasmodels, to analyse the drug release process. TABLE 2 displays the release kinetics of the optimised formulation.

Table 7:

Formulation code	Zero order	First order	Higuchi	Peppas	
	R2	R2	R2	R2	n
F9	0.99	0.8	0.96	0.99	0.8



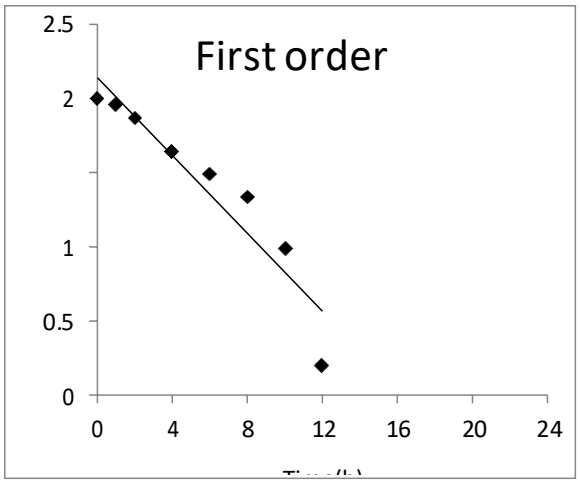


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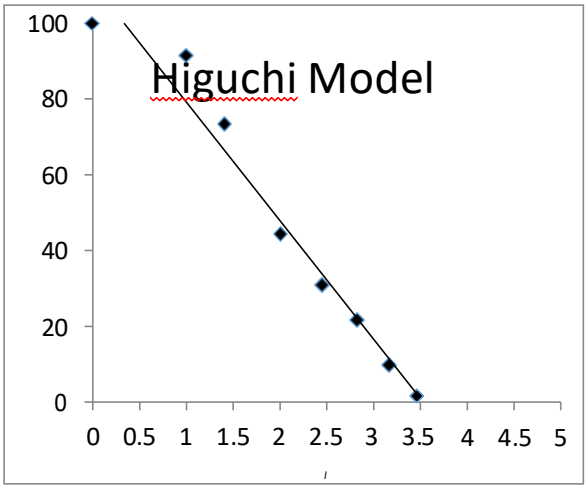
Figure 18: Figure 19:



Stability Studies

There has been no perceivable transition in the physical and chemical properties of the tablet devices of formulation F-9 after three months. The variables that were measured at various times were shown.

Table 8. Results of stability studies of optimized formulation F-9

S.NO	Parameters	Initial	1 month	2 Month	3 month	Limits as per specification
1	400C/75% RH % Release	98.9	98.52	97.79	96.56	Not less than 85 %

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2	400C/75% RH Assay Value	98.9	97.96	96.22	96.00	Not less than 90 % Not more than 110%

RESULT AND DISCUSSION

The current study was carried out in order to develop Viltolarsen solid lipid. Nanospheres Lipid that is solid A variety of polymers were used to create nanospheres containing GMS, Chitosan, PEG6000 SLN, and other additives. The solvent evaporation method was used to create nanospheres. In total, nine formulations were developed and evaluated.

Particle Size Analysis:

The presence of a stabiliser had to have an effect on the particle size of Viltolarsen-fabricated Nanostructures made with different polymers, according to a particle size study. The graphic mean (Mz) and computed surface area (Cs) were used to interpret the particle size investigation results (Table 8). When tried to compare to mean, Graphic Mean provides an average particle size with fewer coarse particles than the diameter of the volume distribution.

Despite the inclusion of the median value, the algorithm considers both smaller and larger molecules, which might also result in a different, and possibly superior, control value. When GMS (F3) was used at 10%, smaller graphic mean (Mz) values were discovered, indicating smaller particles. The formulation F7 with the highest Mz value (275 nm) was observed, indicating larger particles. The polymer accumulation was displayed.

In vitro dissolution:

In vitro dissolution experiments on prepared nanospheres are carried out using a revised solubility process apparatus and a 1% SLS solvent solution. The dissolving rate was discovered to increase linearly to polymer concentration. The improved formulas include (F9). The formulation has reported drug 98.9 in the past 20 hours.

Drug Release Kinetics:

To evaluate the mechanism of drug release, all Sustained formulations' in vitro drug release data have been exposed to a good fit test utilising linear regression analyses in full compliance with order kinetic and the first order reaction equations, Higuchi's, and Korsmeyer-models. Peppa's Table 7 summarises the results of the linear regression, such as the regression coefficients, and figures 8 through 11 depict data plots. As can be seen from the information above, all of the formulations demonstrated 1st order release kinetics ('r' values ranging from 0.900 to 0.965). The

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medication is delivered via a non-fickian diffusion process, as according Higuchi and Peppas' research (n0.5). The kinetic data of the factorial formulations clearly show that the F9 formulation has zero drug release.

CONCLUSION:

Based on the encouraging outcomes of the in drug release tests, the product is suggested for extra in vivo investigations, which might also increase patient compliance. According to the findings, the best formulation is F9, which employs a combination of polymeric materials and Viltolarsen Nanospheres and discharges and over 98.9% of the drug in 20 hours.
According to IR spectroscopic measurements, the improved formulation has no drug-excipient interactions. F9 is a potential Requires Utilization formulation.

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