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Formulation, Optimization And Characterization Of Iguratimod Loaded Nanostructured Lipid Carriers

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Iguratimod is a novel disease-modifying anti-rheumatic drug (DMARD) with proven anti-inflammatory and immunomodulatory effects, making it effective in the management of rheumatoid arthritis (RA). However, its clinical utility is limited by poor aqueous solubility, low oral bioavailability, and rapid metabolism. To address these limitations, this study aimed to develop and optimize nanostructured lipid carriers (NLCs) for the efficient delivery of Iguratimod. NLCs were prepared using a hot homogenization-ultrasonication method and optimized using a Design of Experiments (DoE) approach for key formulation variables including lipid concentration, surfactant concentration, and homogenization parameters. The optimized NLCs were characterized for particle size, polydispersity index (PDI), zeta potential, entrapment efficiency, and in vitro drug release. Morphological studies were performed using transmission electron microscopy (TEM). The optimized formulation exhibited a particle size of ~120 nm, high entrapment efficiency (>85%), and sustained drug release over 24 hours. In vitro release studies revealed a biphasic release pattern with an initial burst followed by sustained release. The study demonstrates that Iguratimod-loaded NLCs offer a promising lipid-based nanocarrier system to improve the solubility, stability, and therapeutic efficacy of Iguratimod for potential use in RA therapy.

Key-words: Iguratimod, Formulation, NLCs.

Introduction

Rheumatoid arthritis (RA) is a chronic systemic autoimmune disorder that leads to progressive joint inflammation, synovial hyperplasia, cartilage destruction, and bone erosion. Despite advancements in pharmacological treatment, achieving effective and safe long-term control of RA symptoms remains a challenge. Iguratimod is an emerging disease-modifying anti-rheumatic drug (DMARD) that inhibits the production of pro-inflammatory cytokines such as TNF-α and IL-6 and suppresses B-cell immunoglobulin production, thereby modulating both innate and adaptive immune responses. Although clinically effective, the therapeutic application of Iguratimod is hindered by its low water solubility, limited oral bioavailability, and extensive first-pass metabolism.

Nanostructured lipid carriers (NLCs), the second generation of lipid nanoparticles, have gained attention for improving the delivery of poorly water-soluble drugs. NLCs are composed of a mixture of solid and liquid lipids stabilized by surfactants, resulting in a less-ordered

internal structure that allows higher drug loading, controlled release, and enhanced stability compared to solid lipid nanoparticles (SLNs). Furthermore, their nanoscale size enables enhanced permeability and bioavailability, making them suitable carriers for oral and parenteral administration of therapeutic agents like Iguratimod.

In this study, we aimed to formulate and optimize Iguratimod-loaded NLCs using a systematic Quality by Design (QbD) approach. The formulation was developed using hot homogenization followed by ultrasonication, and critical parameters affecting the performance of NLCs were evaluated and optimized. The goal was to enhance the solubility and bioavailability of Iguratimod, potentially improving its therapeutic efficacy in the treatment of rheumatoid arthritis.

Material and Methods

Formulation of Iguratimod loaded NLCs

The Iguratimod loaded NLCs were formulated by using ultra-sonication method. For this, mixture of glyceryl monostearate and oleic acid (7:3) in 1-3 % w/v concentration was melted at 70°Cand to this Iguratimod (20 mg) was added. Simultaneously, tween 20 (0.5 - 1.5 % w/v) dispersed in distilled water (20 ml) and heated at the same temperature. Then hot aqueous phase was added to the melted lipid phase and mixture was stirred at 1000 rpm for 30 minutes to get NLC dispersion. This hot dispersion was sonicated at amplitude of 80% for 1 cycle using a probe sonicator and then allowed to settle at room temperature to get drug loaded NLCs.

Optimization of Iguratimod loaded NLCs

The formulation of Iguratimod loaded NLCs was optimized by using Box Behnken design by applying Design-Expert®. The design builds information is shown in table-5.3, list of independent variables is shown in table-5.4, list of response or dependent variables is shown in table-5.5 and experimental designs for NLC formulation is shown in table-5.6. The total lipid concentration(X1), concentration of surfactant (X2) and sonication time (X3) were selected as three independent variables and particle size (R1), polydispersity index (R2) and percentage drug entrapment (R3) were selected as response variables. Further, statistical validity using ANOVA and 3D-response surface plots were established to find the compositions of optimized formulation.

Table- Design builds information using Design-Expert®

Parameters	Remarks
File Version	23.1.6.0
Study type	Response Surface
Design type	Box-Behnken
Design Model	Quadratic
Sub type	Randomized
Runs	13

Table- List of independent variablesselected in experimental design

S. No.	Independent variables	Level of variation		
		Low	Medium	High
1	X1- Total lipid concentration(% w/v)	1	2	3
2	X2- Concentration of surfactant (% w/v)	0.5	1	1.5
3	X3- Sonication time(seconds)	10	15	20

Table- List of response or dependent variables selected in experimental design

S. No.	Response or dependent variables	Units
1	R1- Particle size	nm
2	R2 - Polydispersity index	-
3	R3 - Percentage drug entrapment	%

Table- Box-Behnken experimental design for Iguratimod loaded NLCs

S. No.	Std. Run	Batch No.	Total Lipid Concentration (A) % w/v	Concentration of surfactant (B) % w/v	Sonication time (C) Seconds
1	12	NLCs-1	3	0.5	15
2	8	NLCs-2	1	0.5	15
3	2	NLCs-3	3	1	10
4	1	NLCs-4	2	1	15
5	5	NLCs-5	2	1.5	20
6	10	NLCs-6	1	1	10
7	13	NLCs-7	3	1	20
8	9	NLCs-8	2	1.5	10
9	4	NLCs-9	2	0.5	10
10	7	NLCs-10	1	1.5	15
11	3	NLCs-11	1	1	20
12	6	NLCs-12	2	0.5	20
13	11	NLCs-13	3	1.5	15

Characterization of the Iguratimod loaded NLCs

Particle size and polydispersity index (PDI) analysis

The average particle size and polydispersity index (PDI) of NLCs was determined by using Malvern Zeta sizer at 25°C. All samples were diluted 10 times with double distilled and filtered (0.2µm) water for measurement. Photon correlation spectroscopy (PCS) is a technique employed to determine the mean particle size and PDI.

Zeta potential

It is a parameter highly useful for the assessment of the physical stability of colloidal dispersions. The zeta potential of the NLCs batches was carried out using Malvern Zetasizer. The ξ value for the NLCs was measured for all batches immediately after preparation.

Percent Drug Entrapment:

It was determined by using sephadex G-50 column. For this, NLC dispersion was passed through the saturated sephadex G-50 column and elute was collected. Free Iguratimod was retained in the column, whereas NLCs were eluted out. Eluted fraction was mixed with small amount of DMSO to dissolve lipidic fraction and then diluted with distilled water and analyzed using UV spectrophotometer at 257.16 nm to determine percentage drug entrapment.

In-vitro % drug release study

Cumulative in-vitro % drug release study of optimized Iguratimod loaded NLCs was determined using dialysis bag diffusion technique with phosphate buffer pH 6.8 as release/diffusion medium (500ml). For this, NLC dispersion (equivalent to 10 mg of drug) was placed in dialysis bag which was previously wetted overnight in distilled water, cleaned and sealed from both ends. Then dialysis bag (already filled with NLC dispersion) was plunged in the receptor compartment containing the diffusion medium which was stirred at 50rpm at 37±0.5°C. Samples (5ml) from diffusion medium were withdrawn at regular time intervals and same volume was replaced with fresh medium to maintain the volume. Then samples were analyzed using U.V. visible spectrophotometer to determine the concentration of Iguratimod and cumulative in-vitro % drug release calculated and plotted against time.

Shape and surface morphology using transmission electron microscopy (TEM)

TEM analysis of the optimized NLC formulations was carried out to understand the shape and surface morphology. For this, a drop of Iguratimod loaded NLC dispersion with 0.01% of phosphotungstic acid was placed on a carbon film coated on a copper grid. Then copper grid was placed into sample holder and fixed in vacuum chamber and images were recorded at 80 kV using TEM.

Results and Discussion

Formulation of Iguratimod loaded NLCs

Iguratimod loaded NLCs were prepared using ultr-asonication method. The resultant NLC dispersion was sonicated for reducing its particle size.

Optimization of Iguratimod loaded NLCs

Formulation of Iguratimod loaded NLCs was optimizedusing Box Behnken design. There were total 13 runs as per experimental design and were prepared in randomized manner. The effects of different levels of independent variables on the response variables were investigated. As Design suggested linear model as the best fit for all three response variables. The effect of independent variables over the response variables is shown in table-6.3. Further, statistical validity were performed using ANOVA test to create R^2, adjusted R^2, predicted R^2, standard deviation and % coefficient of variance.

Table- Effect of independent variables on response variables

Batch No.	Independent variable			Response variables		
	(X1) % w/v	(X2) % w/v	(X3) seconds	(R1)	(R2)	(R3)
NLCs-1	3	0.5	15	157.87	0.245	76.45
NLCs-2	1	0.5	15	149.53	0.216	71.43
NLCs-3	3	1	10	152.18	0.239	79.29
NLCs-4	2	1	15	150.71	0.231	73.44
NLCs-5	2	1.5	20	148.29	0.225	73.45
NLCs-6	1	1	10	146.94	0.217	71.41
NLCs-7	3	1	20	151.16	0.244	79.29
NLCs-8	2	1.5	10	149.07	0.228	76.59
NLCs-9	2	0.5	10	150.26	0.232	74.33
NLCs-10	1	1.5	15	144.76	0.211	71.76
NLCs-11	1	1	20	143.19	0.213	70.57
NLCs-12	2	0.5	20	147.53	0.227	75.41
NLCs-13	3	1.5	15	150.04	0.242	77.64

Effect of independent variables on particle size

The effect of independent variables on the particle sizewas determined for NLCs and recorded in form of 3D surface plot as shown in figure-6.14. Results suggested decreased value of particle size with the increase in the concentration of surfactant and sonication time. Particle size was found to be slightly increased on increasing the total lipid concentration. The optimized values of particle size were found to be 143.7 nm. The summary of statistical data of ANOVA test forparticle sizeis shown in table-6.4.

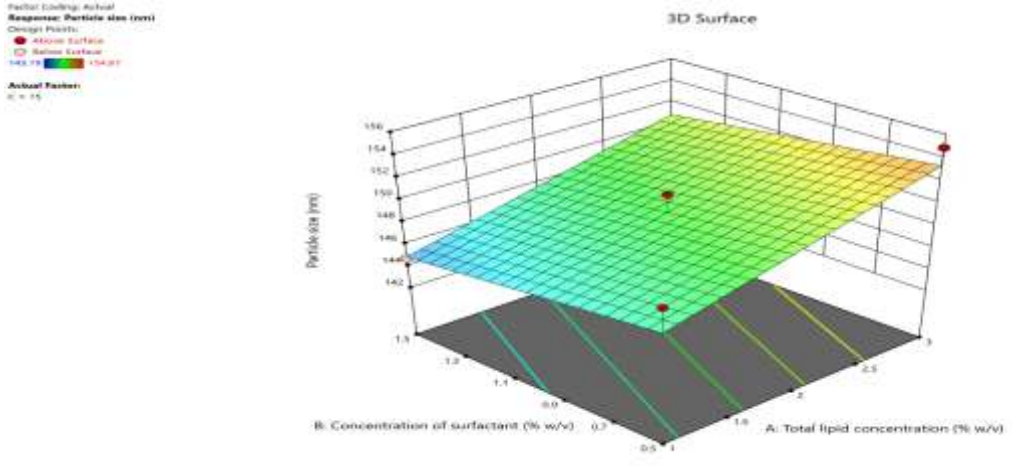

Figure- 3D surface plot of effect of independent variables on particle size

Table- Summary of statistical data of ANOVA for particle size

Formulations	R^2	Adjusted R^2	Predicted R^2	SD	% CV
NLCs	0.8112	0.74822	0.6062	1.54	1.04

Effect of independent variables on polydispersity index

The effects of independent variables on the polydispersity index were determined for NLCs and recorded in form of 3D surface plot as shown in figure-6.15. Results suggested decreased value of polydispersity index with the increase in the concentration of surfactant and sonication time. The change in concentration of total lipid had not shown any significant variation over the polydispersity index of the NLCs. The optimized values of polydispersity index were found to be 0.211. The summary of statistical data of ANOVA test for polydispersity indexis shown in table-6.5.

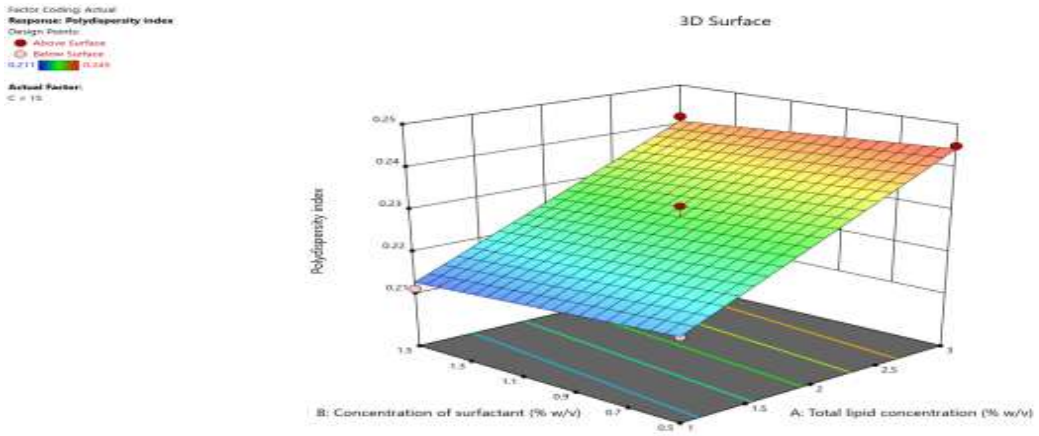

Figure- 3D surface plot of effect of independent variables on polydispersity index

Table- Summary of statistical data of ANOVA for polydispersity index

Formulations	R^2	Adjusted R^2	Predicted R^2	SD	% CV
NLCs	0.9722	0.9722	0.9630	0.0023	0.9947

Effects of independent variables on percentage drug entrapment

The effects of independent variables on the percentage drug entrapmentwere determined for NLCs and recorded in form of 3D surface plot as shown in figure-6.16. Results suggested increased value of percentage drug entrapmentwith the increase in the total lipid concentration and increased surfactant concentration. However, the optimum combination of both also affect the percentage drug entrapmentsignificantly. The optimized values of percentage drug entrapment were found to be 73.24%. The summary of statistical data of ANOVA test for percentage drug entrapmentis shown in table-6.6.

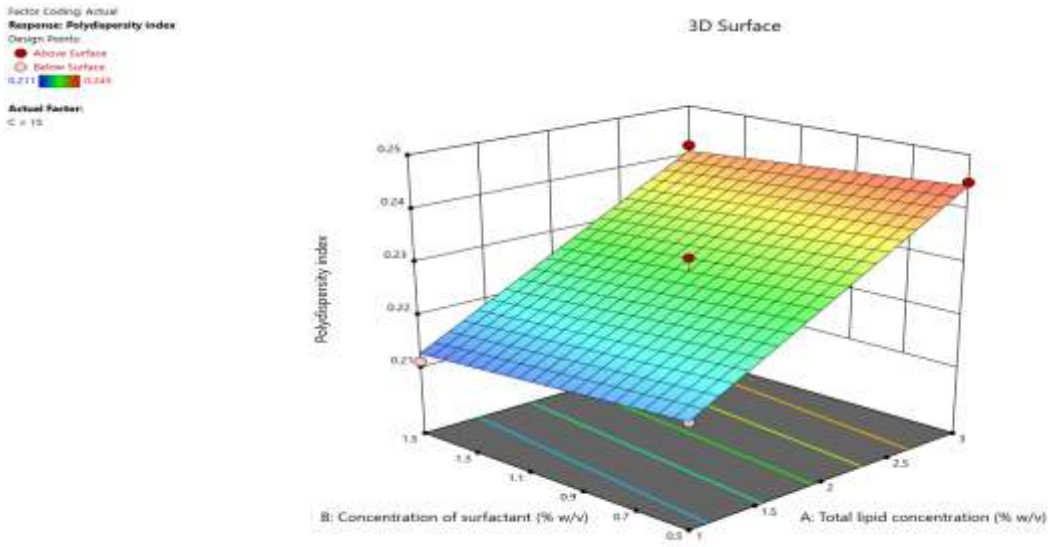

Figure- 3D surface plot of effect of independent variables on percentage drug entrapment

Table- Summary of statistical data of ANOVA for percentage drug entrapment

Formulations	R^2	Adjusted R^2	Predicted R^2	SD	% CV
NLCs	0.8854	0.8472	0.7621	1.18	1.57

Based on the results and observations of formulation optimization using Box-Behnken design on Design-Expert®, the optimized values of independent and dependent or response variables were obtained.

Further, formulation of NLCs were validated using optimized concentration of independent variable and validated results for NLCsare shown in table-6.7. Validated values of response variables were found to be close to that of optimized values depending on the statistical analysis.

Table- Validated values of independent variables and response variables for NLCs

Type of Variable	Variables	Optimized Value	Validated Value (n=3)
Independent	Total lipid concentration (% w/v)	1.834	1.834
	Concentration of surfactant (% w/v)	1.5	1.5
	Sonication time (Seconds)	20	20
Response or Dependent	Particle size (nm)	146.33	143.7
	Polydispersity index	0.223	0.211
	Percentage drug entrapment (%)	73.99	73.24

Characterization of the Iguratimod loaded NLCs

Particle size and polydispersity index (PDI) analysis

The particle size and PDI of the optimized formulation was found to be 143.7 nm and 0.211. The particle size and PDI of all the batches of Iguratimod loaded NLCs are shown in table-6.8. Particle size distribution image of optimized formulation is shown in figure 6.17. Results revealed the presence of low size monodipersed particles in NLC dispersion.

Table- Particle size and PDI values of Iguratimod loaded NLCs (n=3)

S. No.	Batch No.	Particle Size (Z avg in nm ± SEM)	PDI
1	NLCs-1	157.87±0.2	0.245±0.03
2	NLCs-2	149.53±0.3	0.216±0.09
3	NLCs-3	152.18±0.2	0.239±0.06
4	NLCs-4	150.71±0.4	0.231±0.08
5	NLCs-5	148.29±0.1	0.225±0.05
6	NLCs-6	146.94±0.3	0.217±0.04
7	NLCs-7	151.16±0.5	0.244±0.07
8	NLCs-8	149.07±0.2	0.228±0.09
9	NLCs-9	150.26±0.7	0.232±0.03
10	NLCs-10	144.76±0.5	0.211±0.11
11	NLCs-11	143.19±0.3	0.213±0.05
12	NLCs-12	147.53±0.8	0.227±0.09
13	NLCs-13	150.04±0.4	0.242±0.08
14	Optimized	142.39±0.2	0.212±0.05

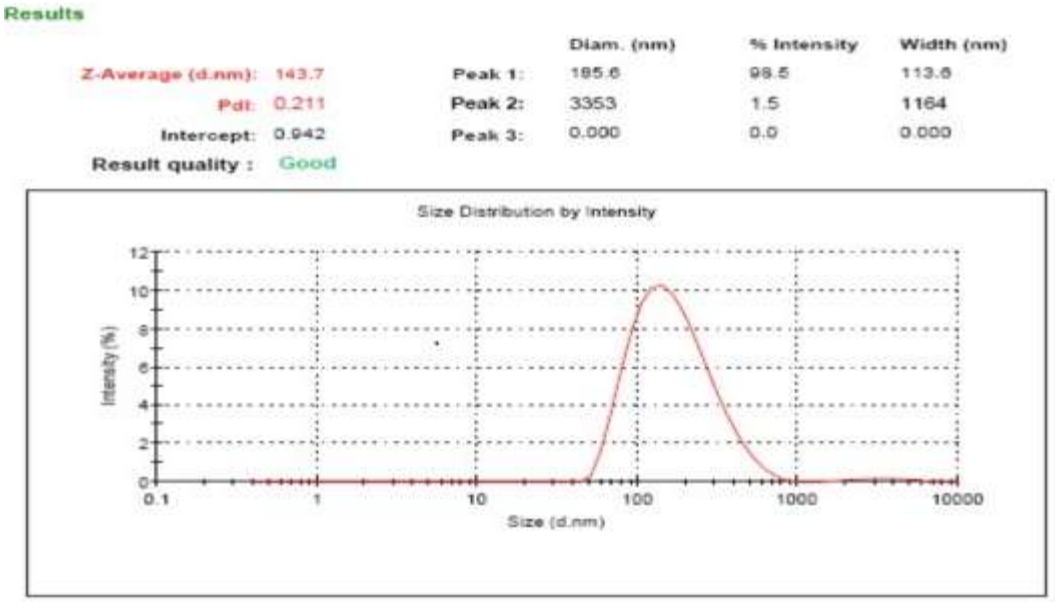

Figure- 6Particle size distribution of optimized Iguratimod loaded NLCs

Zeta potential

The zeta potential of the optimized formulation was found to be -19.1 mV. The zeta potential of all the batches of Iguratimod loaded NLCs are shown in table-6.9. Zeta size image of optimized formulation is shown in figure 6.18. High value of zeta potential indicated the good stability of the NLC formulation.

Table- Zeta potential of Iguratimod loaded NLCs (n=3)

S. No.	Batch No.	Zeta potential (mV±SEM)
1	NLCs-1	-18.4±0.1
2	NLCs-2	-17.6±0.2
3	NLCs-3	-19.5±0.5
4	NLCs-4	-14.7±0.4
5	NLCs-5	-18.2±0.3
6	NLCs-6	-17.5±0.2
7	NLCs-7	-18.8±0.4
8	NLCs-8	-17.9±0.2
9	NLCs-9	-18.5±0.4
10	NLCs-10	-19.1±0.1
11	NLCs-11	-18.9±0.3
12	NLCs-12	-18.3±0.3

13	NLCs-13	-17.8±0.4
14	Optimized	-19.1±0.3

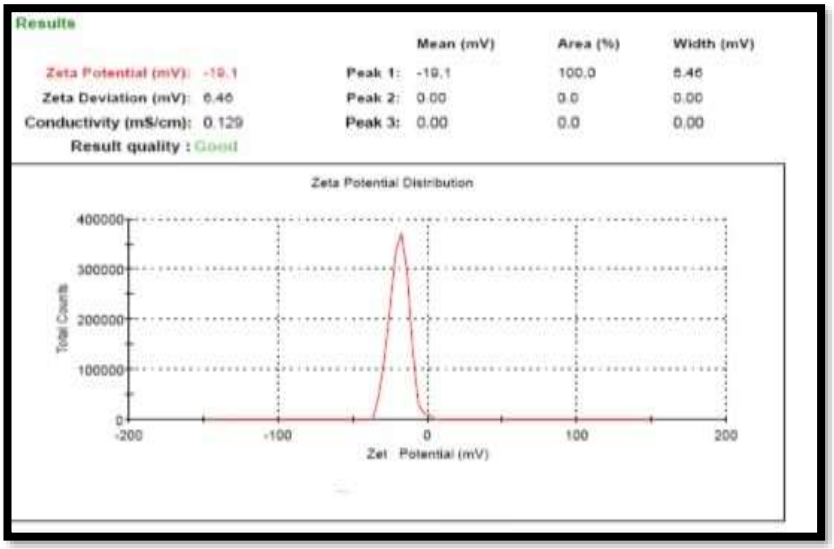

Figure - Zeta potential of optimized Iguratimod loaded NLCs

Percentage drug entrapment

The percentage drug entrapment of the optimized formulation was found to be 73.24±1.23%. The percentage drug entrapment of all the batches of Iguratimod loaded NLCs are shown in table-6.10. results indicated the high drug entrapment into the lipidic core of the NLCs.

Table- Percentage drug entrapment of Iguratimod loaded NLCs (n=3)

S. No.	**Batch No.**	**Percentage drug entrapment** (%±SEM)
1	NLCs-1	71.43±1.04
2	NLCs-2	79.29±1.37
3	NLCs-3	73.44±0.98
4	NLCs-4	73.45±1.26
5	NLCs-5	71.41±1.16
6	NLCs-6	79.29±1.23
7	NLCs-7	76.59±1.18
8	NLCs-8	74.33±1.51
9	NLCs-9	71.76±1.23
10	NLCs-10	70.57±1.73

11	NLCs-11	75.41±0.94
12	NLCs-12	77.64±1.07
13	NLCs-13	71.43±1.38
14	Optimized	74.39±1.01

In-vitro % drug release study

Cumulative in-vitro % drug release study of optimized Iguratimod loaded NLCs was found to be 83.18±1.36 % upto 30 hours and data and drug release plot is shown in table 6.11 and figure 6.19 respectively. Results of the study revealed the slow and sustained release of Iguratimod from the NLCs.

Table- Cumulative in-vitro % drug release data of optimized Iguratimod loaded NLCs (n=3)

Time (Hours)	**Cumulative in-vitro % drug release** (%±SEM)
0	0
1	5.20±0.32
2	9.33±1.10
3	13.11±1.22
4	18.22±1.45
5	20.72±1.56
6	23.84±1.81
7	28.65±1.32
8	33.58±1.11
9	39.19±1.48

10	44.83±1.71
24	75.10±1.48
30	83.18±1.36

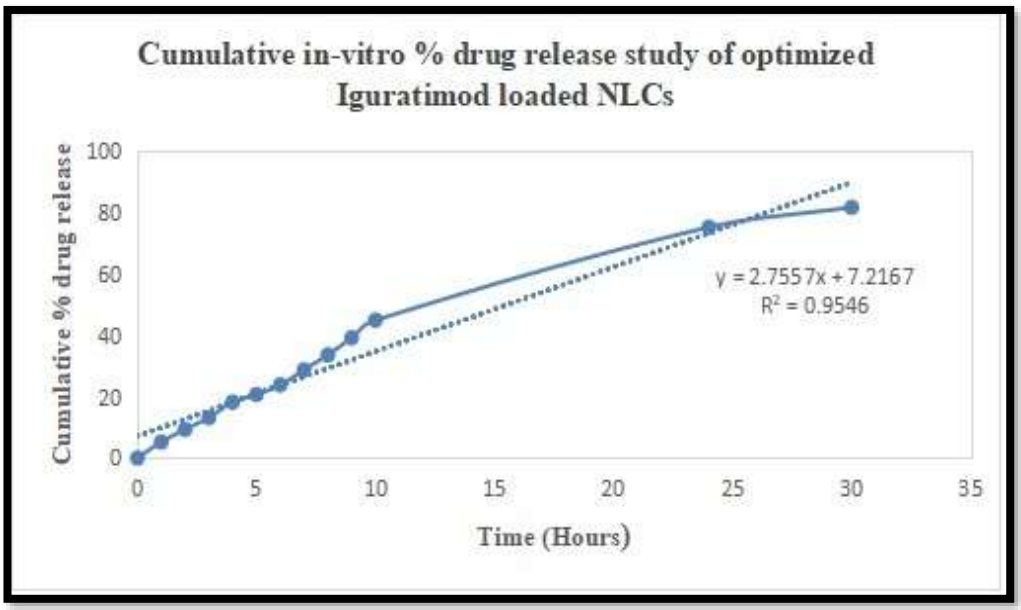

Figure - Cumulative n-vitro % drug release data of optimized Iguratimod loaded NLCs
Shape and surface morphology using transmission electron microscopy (TEM)
TEM image of Iguratimod loaded NLCs is shown in figure-6.20. It indicated the smooth surface and spherical shape of the particle with size range of upto 140-150 nm.

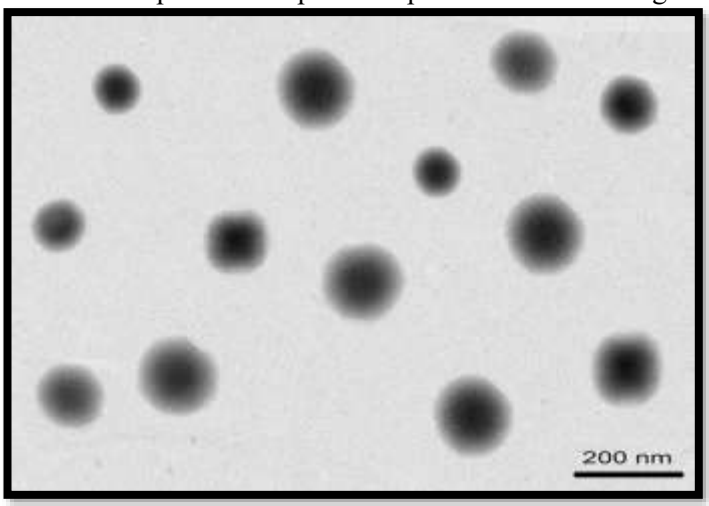

Figure - 6TEM image of optimized Iguratimod loaded NLCs

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