RESEARCH ARTICLE

Formulation, Optimization and Characterization of Preliminary Screening of Materials Iguratimod Loaded NLCs

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ABSTRACT
Rheumatoid arthritis is treated with iguratimod. Disease-modifying anti-rheumatic medication iguratimod. Rheumatoid arthritis inflammation is reduced by improving aberrant immune response and decreasing inflammation-causing chemicals. This paper optimised Iguratimod-loaded NLC formulation using Box Behnken design. 13 randomised runs were produced for the experiment. This paper examined how independent variable levels affected response variables.
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INTRODUCTION

Nanotechnology is crucial to innovative drug delivery. Solid lipid nanoparticles are a promising nanotechnology for targeted drug delivery and clinical use. New therapies can be developed due to its size-dependent properties. Its capacity to encapsulate drugs in lipid matrix provides a novel precursor for enhanced drug targeting. Researchers have focused on developing site-oriented, safe, and effective formulations for solid lipid nanoparticles due to their unusual features.[1] Surfactant and stabilisers keep these lipids solidified at room and body temperature. Iguratimod (IGU), a disease-modifying anti-rheumatic medication (DMARD), treats rheumatoid arthritis.

MATERIAL AND METHODS

Initial Material Screening

Choice of Solid Lipid

This was based on Iguratimod's partitioning into melting lipid and water phases. Using a magnetic stirrer and hot plate, 200mg of glyceryl monostearate, palmitostearate, and behenate were heated to 60-70°C in a glass vial. Iguratimod (10mg) was added to the melting lipid phase. Drug dissolved in melting fat. Add 5ml of distilled water heated to the same temperature. After 30 minutes of stirring at 60-70°C, the mixture was cooled to room temperature. The mixture was filtered with Whatman paper. Then a UV spectroscophotometer at 257.1 nm measured drug partitioning in filtrate.

Choice of Liquid Lipid

Iguratimod was added to liquid lipid (2ml each of Capmul MCM, Oleic acid, and Labrafil) in a vial and shaken at room temperature on a mechanical shaker until undissolved in each beaker. Ultracentrifuged vial contents for 10 minutes at 10000 rpm. A U.V. visible spectroscophotometer at 257.1 nm measured drug partitioning in sand dissolved in phosphate buffer pH 6.8. 6-9

Choice of Surfactant

An excess of 20 mg of Iguratimod was put to a vial containing 1 ml of surfactants (tween 20, tween 80, and PEG 400) to test its solubility. A vortex shaker was used to attain equilibrium in the mixes after 72 hours at 25°C. To remove excess Iguratimod, the solutions were centrifuged at 10000 rpm for 5 min and filtered. U.V. visible spectroscopy determined Iguratimod in the filtrates.

Iguratimod-Loaded NLC Formulation

Ultra-sonication created Iguratimod-loaded NLCs. Iguratimod (20 mg) was added to a melted mixture of glyceryl monostearate and oleic acid (7:3) at 1-3 % w/v concentration. Tween 20 (0.5–1.5%) dispersed in distilled water (20 ml) and heated at the same temperature. To get NLC dispersion, heated aqueous phase was added to melted lipid phase and agitated at 1000 rpm for 30 minutes. Drug-loaded NLCs were obtained by sonicating this hot dispersion at 80% amplitude for 1 cycle using a probe sonicator and letting it settle at room temperature.[2,3]

Optimising Iguratimod-Loaded NLCs

Box Behnken design and Design-Expert® optimised Iguratimod-loaded NLC formulations. Table-1 shows design builds, table-2 lists independent variables, table-3

Table 1: 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

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lists response or dependent variables, and table-4 lists experimental designs for NLC formulation. The independent variables were total lipid concentration (X1), surfactant concentration (X2), and sonication time (X3), whereas the response variables were particle size (R1), polydispersity index (R2), and percentage drug entrapment (R3). To determine optimised formulation compositions, ANOVA and 3D-response surface plots showed statistical validity.[4,5]

Characterization of the Iguratimod Loaded NLCs

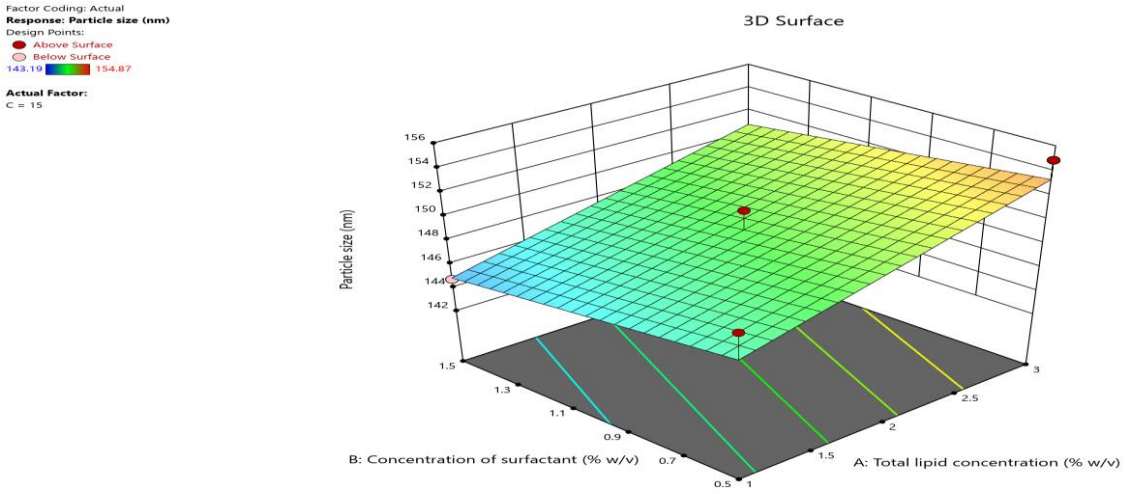

Figure 1: Surface plot of effect of independent variables on particle size

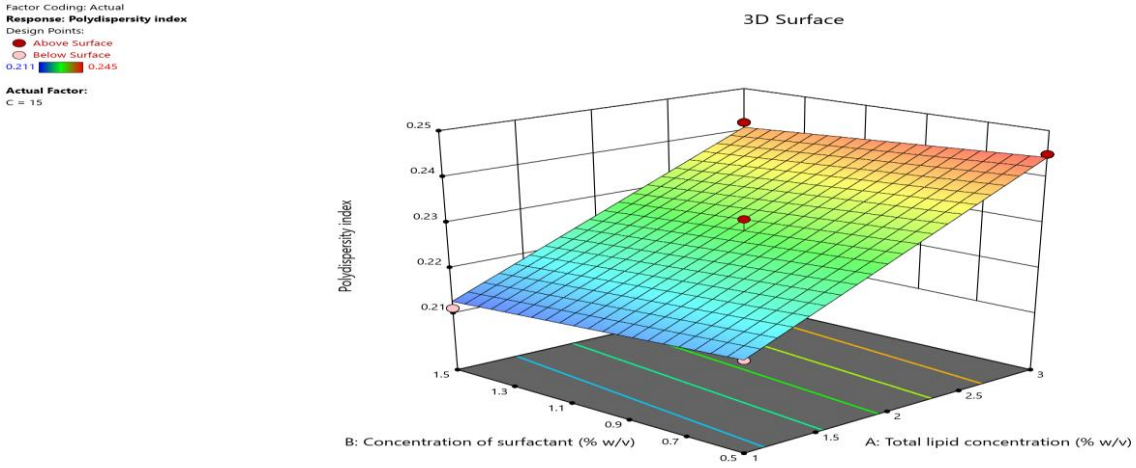

Figure 2: 3D surface plot of effect of independent variables on polydispersity index

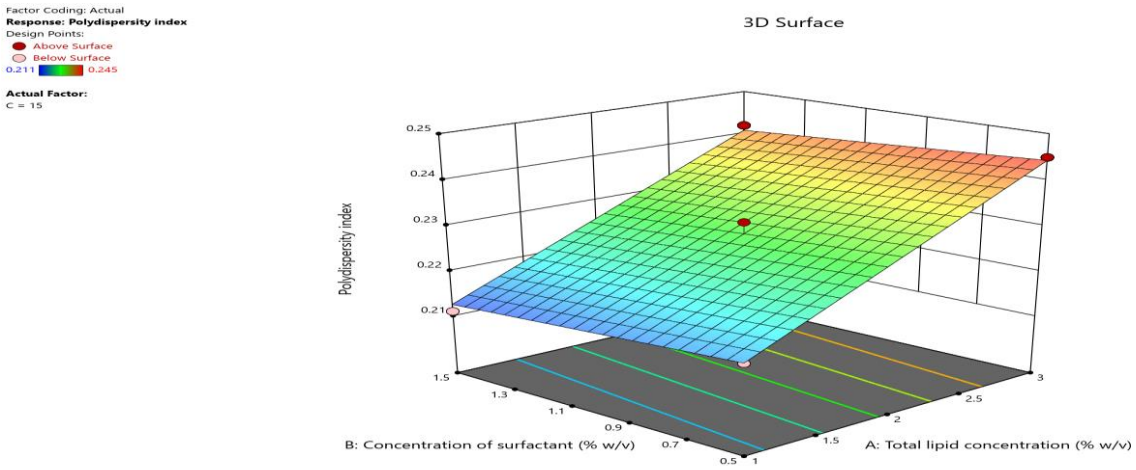

Figure 3: 3D surface plot of effect of independent variables on percentage drug entrapment

Table 2: List of independent variables selected 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 3: 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	%

Drug loaded NLC were evaluated for particle size using using Malvern Zeta sizer at 25℃. 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 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. *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

Figure 4: Particle size distribution of optimized Iguratimod loaded NLCs

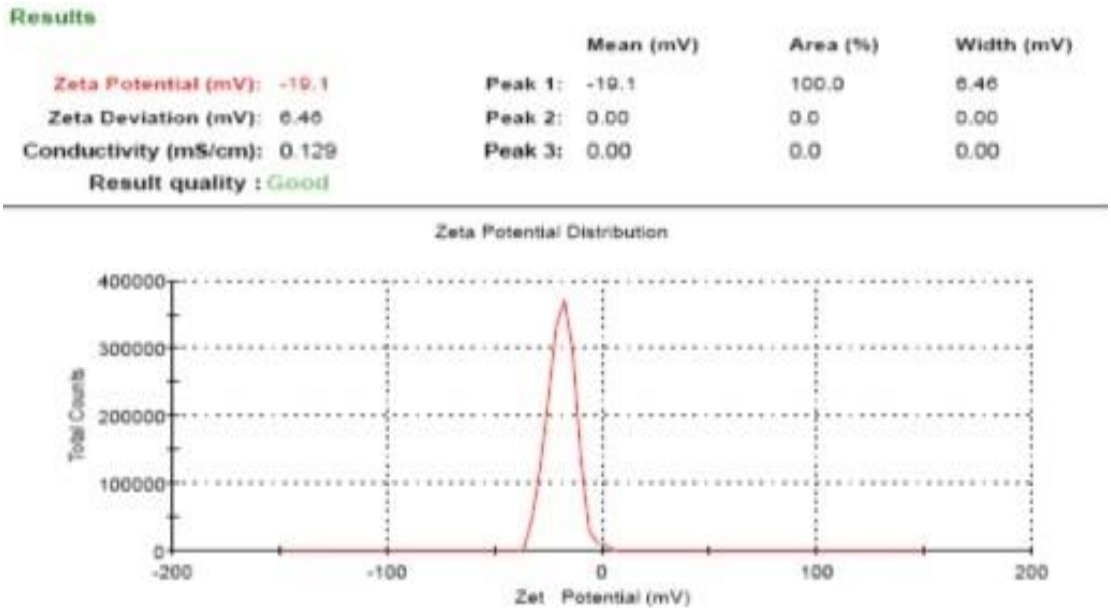

Figure 5: Zeta potential of optimized Iguratimod loaded NLCs

Table 4: 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

Table 5: 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

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. TEM analysis of the optimized NLC formulations was carried out to understand the shape and surface morphology.[5,6]

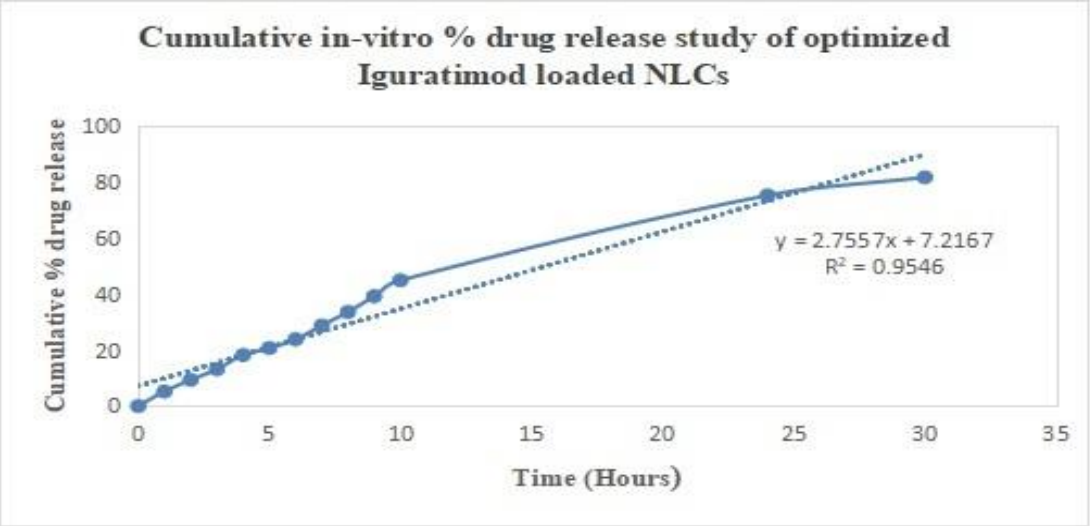

Figure 6: Cumulative *n-vitro* % drug release data of optimizedIguratimod loaded NLCs

Table 6: 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

Table 7: Characterization of Iguratimod loaded NLCs (n=3)

S. No.	Batch No.	Particle Size (Z avg in nm ± SEM)	PDI	Zeta potential (mV±SEM)	Percentage drug entrapment (%±SEM)
1	NLCs-1	157.87±0.2	0.245±0.03	-18.4±0.1	71.43±1.04
2	NLCs-2	149.53±0.3	0.216±0.09	-17.6±0.2	79.29±1.37
3	NLCs-3	152.18±0.2	0.239±0.06	-19.5±0.5	73.44±0.98
4	NLCs-4	150.71±0.4	0.231±0.08	-14.7±0.4	73.45±1.26
5	NLCs-5	148.29±0.1	0.225±0.05	-18.2±0.3	71.41±1.16
6	NLCs-6	146.94±0.3	0.217±0.04	-17.5±0.2	79.29±1.23
7	NLCs-7	151.16±0.5	0.244±0.07	-18.8±0.4	76.59±1.18
8	NLCs-8	149.07±0.2	0.228±0.09	-17.9±0.2	74.33±1.51
9	NLCs-9	150.26±0.7	0.232±0.03	-18.5±0.4	71.76±1.23
10	NLCs-10	144.76±0.5	0.211±0.11	-19.1±0.1	70.57±1.73
11	NLCs-11	143.19±0.3	0.213±0.05	-18.9±0.3	75.41±0.94
12	NLCs-12	147.53±0.8	0.227±0.09	-18.3±0.3	77.64±1.07
13	NLCs-13	150.04±0.4	0.242±0.08	-17.8±0.4	71.43±1.38
14	Optimized	143.70±0.3	0.211±0.06	-19.1±0.2	73.24±1.23

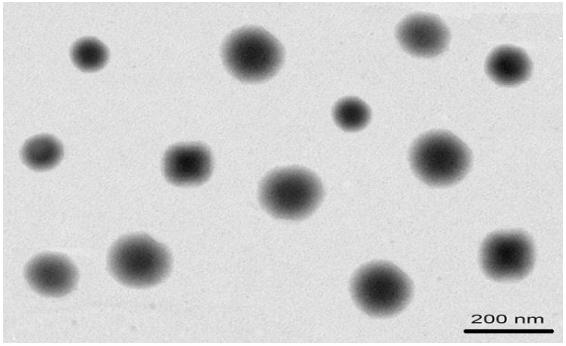

Figure 7: TEM image of optimized Iguratimod loaded NLCs

RESULTS AND DISCUSSION

Iguratimod loaded NLCs were prepared using ultr-asonication method. The resultant NLC dispersion was sonicated for reducing its particle size. 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-5. Further, statistical validity were performed using ANOVA test to create R^2, adjusted R^2, predicted R^2, standard deviation and % coefficient of variance.

Characterization of the Iguratimod Loaded NLCs

The characterization parameters of the formulated were mentioned in table 7.

Table 8: Cumulative *in-vitro* % drug release data of optimizedIguratimod loaded NLCs (n=3)

Time (Hours)	Cumulative *in-vitro* % drug release (%±SEM)
0	0
1	5.21±0.92
2	9.36±1.35
3	13.17±1.26
4	18.24±1.64
5	20.73±1.15
6	23.86±1.91
7	28.79±1.47
8	33.62±1.05
9	39.24±1.52
10	44.97±1.81
24	75.26±1.37
30	81.74±1.68

CONCLUSION

The present study showed that SLNs, a versatile technology, can improve the biopharmaceutics properties of poorly water-soluble drugs like Iguratimod (BCS class II) and open new avenues for drug formulation with low aqueous solubility and high permeability. The SLN method was used to improve oral bioavailability. Optimisation of Drug [IGU]-SLN was done. Optimisation of drug-SLN included decreased particle size, minimal polydispersity index, sustained drug release from SLN, lower surfactant concentration, better drug solubilisation in minimum lipid, and higher bioavailability. SLN entraps medicines, allowing controlled release and prolonged release of both substances. The drug release profile in-vitro reveals the

highest release for IGU-loaded SLNs (81.74±1.68 in 30 hours). The release data shows SLNs improve medication.

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