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Research

Preparation and Evaluation of Aceclofenac and Piperine Loaded Solid Lipid Nanoparticles Using Box- Behnken Design

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Abstract

The Aim of present study was preparation and evaluation of loaded Solid Lipid Nanoparticles using Box- Behnken Design. The three-component, three-level Box Behnken design, which was ideal for examining quadratic response surfaces and creating polynomial models with Design-Expert software (Version 13), was used to explore the influence of factors on solid lipid nanoparticles performance and features. The SLNs were prepared by Ultra sonication method using Vanaspati ghee and VCO as lipid, Glyceryl monostearate used as an emulsifier, Polysorbate 80 as surfactant and emulsifier, Carbopol 940 as polymer. The SLNs were evaluated for its Particle size, Polydispersity Index, Zeta potential, Entrapment Efficiency and In vitro % drug release. The FTIR studies revealed no chemical interaction between the drug molecule and polymers and found that drug was compatible with used polymer. Aceclofenac and Piperine loaded SLN formulation predicted by software consisted of 250 mg lipid, 600µl surfactant and 250 mg extract which on characterization studies found to have mean particle size of 85.5±8.2 nm, PDI of 0.342±0.06, zeta potential -13.5 mV, % EE (Ace) 82.73±2.89%, and %EE (Pip) 84.01±3.92%, which were in close agreement with the predicted values. The developed optimized formulation exhibited sustained drug release (Ace and Pip) for 24 h duration to the extent of 85.27±5.6% and 56.04±3.68% it was concluded that the Aceclofenac And Piperine loaded SLN based gel formulation containing carbopol was suitable for topical application and shows much better result in Rheumatoid arthritis (RA),

Keywords: Nanoparticles, Aceclofenac, Piperine, Design-Expert software, Ultra sonication, Carbopol 940, Glyceryl monostearate

INTRODUCTION

Topical delivery includes site-specific drug delivery, reducing systemic toxicity, increasing patient compliance, increasing treatment efficacy, and improving bioavailability. On the other hand, topical delivery of anti-rheumatoid drugs can cause adverse skin reactions like allergic reactions and itching.

Solid lipid nanoparticles are at the forefront of the quickly growing field of nanotechnology and have several potential uses in clinical treatment, medication delivery, research, and a variety of other fields of science. Lipid nanoparticles provide the potential to create novel therapies because of their distinct size-dependent characteristics. Medication delivery technology has a new prototype thanks to the capacity to combine pharmaceuticals into Nano carriers, which might be applied to secondary and tertiary levels of drug targeting.

Aceclofenac is a non-steroidal anti-inflammatory drug (NSAID) used to treat rheumatoid arthritis and osteoarthritis by reducing pain and inflammation. But it also has negative effects on the liver, renal, platelet, and gastrointestinal systems when taken chronically. Additionally, the medication has a very short biological half-life (half-life: 3–4 hours), therefore a dosage of 100 mg is taken twice a day orally. Piperine (C17H19NO3) is an alkaloid primarily found in the plants Piper and P. nigrum. It is grown in India, Brazil, and Indonesia's tropical regions. This chemical has numerous advantages including antioxidant, anticancer, antihypertensive, analgesic, antipyretic, anti-inflammatory, hepato-protective, antibacterial, immunomodulatory, antifungal, etc. It dramatically decreases ankle joint inflammation and arthritic symptoms in mice (topically). It also serves as a bio enhancer when used in conjunction with other nutraceutical agents. To achieve prolonged drug release and greater therapeutic advantages, the formulation of a solid-lipid Nano particulate system as a carrier for aceclofenac combined with piperine may be advantageous.

MATERIALS AND METHOD

Materials

Aceclofenac was obtained as gift- sample from Luex Pharmaceuticals Pvt. Ltd., Mumbai and carbopol 934, Tween 80 of pharmaceutical grade were procured from Sunchem Industry Navi Mumbai, Maharashtra. Black Pepper, Vanaspati ghee, Virgin coconut oil procured from Local market, Disodium hydrogen phosphate, Potassium hydrogen phosphate procured from Thermo Fisher Scientific.

Experiemental method

Pre-formulation Studies

Pre-formulation studies were conducted to establish the drug's identification, physiochemical factor, kinetics rate profile, physical features, and compatibility with excipients.

Method for preparation of aceclofenac and piperine- loaded solid lipid nanoparticles

The three-component, three-level Box Behnken design, which was ideal for examining quadratic response surfaces and creating polynomial models with Design-Expert software (Version 13), was used to explore the influence of factors on solid lipid nanoparticles performance and features. The following are independent variables Amount of lipid (mg), Amount of Tween 80 (µl), Amount of black pepper extract(mg) and dependent variables Particle size (nm), The polydispersity index (P.I.), Zeta potential (mV), Entrapment efficiency Aceclofenac (%), Entrapment efficiency Piperine (%). In-vitro cumulative drug release that were chosen to be researched using the Box-Behnken Design based on preliminary investigations and literature review.

Table 1: Formulation Protocol Using Box Behnken Design

Formulation code	Lipid (mg) (A)	Surfactant (ml) (B)	Extract (mg) (C)
F1	200	600	200
F2	300	600	200
F3	200	700	250
F4	250	600	250
F5	250	700	200
F6	250	600	250
F7	300	500	250
F8	250	700	300
F9	250	500	200
F10	250	600	250
F11	200	500	250

F12	250	600	250
F13	250	600	250
F14	250	500	300
F15	200	600	300
F16	300	700	250
F17	300	600	300

Preparation of Solid Lipid Nanoparticles

The SLNs were prepared by Ultrasonication method. The required amount of lipids (Vanaspati ghee: GMS {1:1} & VCO), extract and Aceclofenac were taken in a beaker and in another beaker needed amount of surfactant solution was taken. Both the lipid phase and aqueous phase were heated simultaneously. When the lipid was melted, the aqueous phase was added slowly to the lipid phase with continuous high-speed stirring for 10 minutes with gradual cooling. The mixture was then sonicated for one cycle of 5 mins with an on-time of 1sec and off-time of 2secs. A pinch of sodium azide (preservative) was added to the formulation.

Skin irritation study

The Draize patch test for rats was used to test the formulated formulations for primary cutaneous irritation. The animals in this study were divided into two groups, each with n=3 animals. For assessing the SLN irritancy potential, a skin irritancy test was performed on rats (180–200g). For up to three days, the rats were fed a conventional meal. Depilatory cream was used to remove hairs from the rat's dorsal side.

The animals are divided into two groups (n = 3):

Group I: Control group

Group II: Carbopol gel base group

Group III: Carbopol SLN gel group

Their respective groups were dispersed on shaved skin across a 2 cm^2 area. The rats were labelled and placed individually in their respective cages after treatment, and cutaneous responses were documented at 30 minutes, 24 hours, 48 hours, and 72 hours. Skin responses in terms of mean erythema, edema scores, and changes in skin texture were noted based on their severity.

Table 2: Scoring of Skin Reaction (Erythema/Edema) Observations

Observations	Scoring
No reaction	0
Slight reaction with barely perceptible light pink	1
Moderate reaction with dark pink	2
Moderate to severe reaction with light red	3
Severe reaction with extreme redness	4

Skin irritation Experimental Protocol Design Chart Skin irritation study

- **No. of groups**: - 03 (n=3)
- **Animals: -** Wistar rats, Female
- **Weight: -** 180-200 gms

Table 3: Skin irritation study

Days	Control group	Carbopol group gel base	Carbopol group SLN gel
Day 0	Hair Removal	Hair Removal	Hair Removal
Day 1	No Treatment	Application of gel base Inspection after 30 mins	Application of formulation Inspection after 30 mins
Day 2	Inspection after 24 hrs	Inspection after 24 hrs	Inspection after 24 hrs
Day 3	Inspection after 48 hrs	Inspection after 48 hrs	Inspection after 48 hrs
Day 4	Inspection after 72 hrs	Inspection after 72 hrs	Inspection after 72 hrs
Day 5	Evaluation of infection	Evaluation of infection	Evaluation of infection

RESULTS AND DISCUSSION

Preformulation studies:

a) Organoleptic properties

Table 4: Description of the Aceclofenac and Black Pepper Extract

S. No.	Test	Observation (Aceclofenac)	Black Pepper Extract
1.	Colour	White	Dark green
2.	Odor	Odorless	Sharp, yet slightly sweet.
3.	Physical appearance	Crystalline powder	Semi solid

b) Melting point

Table 5: Melting Point of the drug

S. No.	Drug	Observation
1.	Aceclofenac	149-153 ℃

c) Solubility study (Aceclofenac): As per IP.

d) Calibration Curve of Aceclofenac

Table 6: Absorbance of different dilutions of Aceclofenac (λmax= 275nm)

S. No.	Concentration(µg/ml)	Absorbance
1	2	0.0325
2	4	0.0857
3	6	0.1308
4	8	0.1766
5	10	0.2207

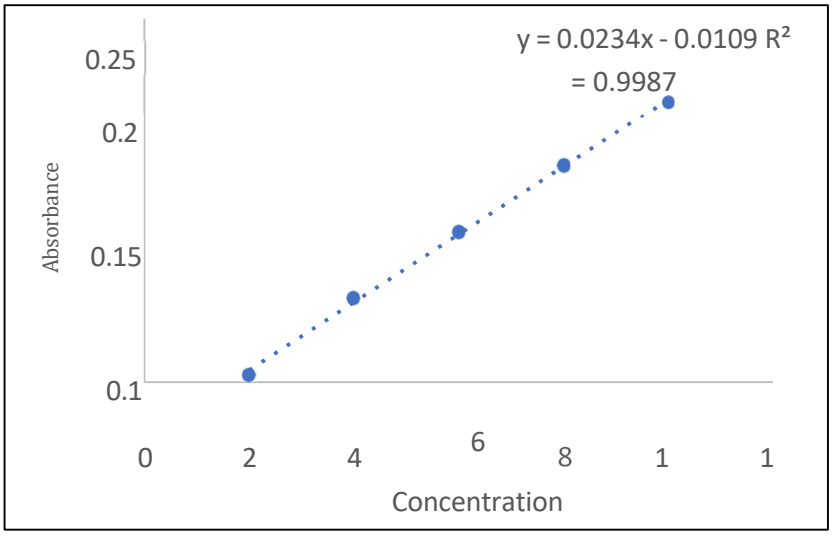

Fig 1: Calibration curve of Aceclofena

a) Calibration Curve of Piperine

Table 7: Absorbance of different dilutions of Piperine (λmax= 342nm)

S. No.	Concentration(µg/ml)	Absorbance
1	2	0.1148
2	4	0.2378
3	8	0.5261
4	12	0.8248
5	16	1.1797
6	20	1.5453

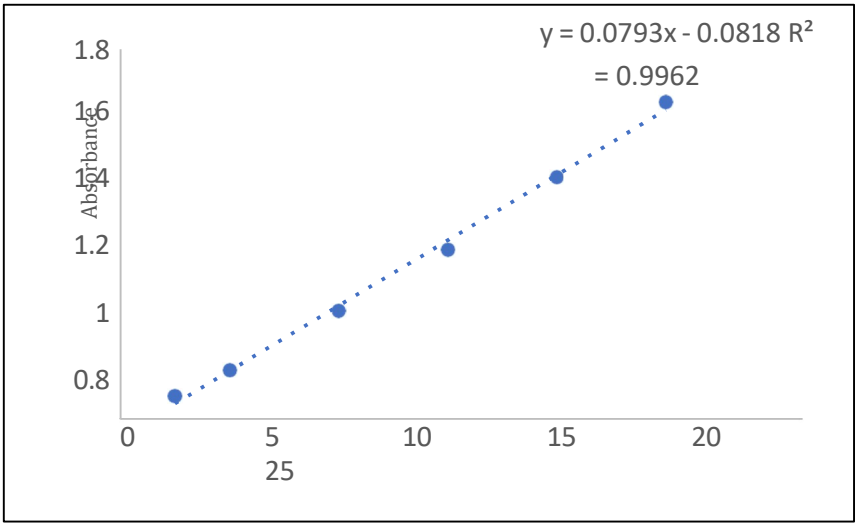

Fig 2: Calibration curve of Piperine

(f) FTIR Study

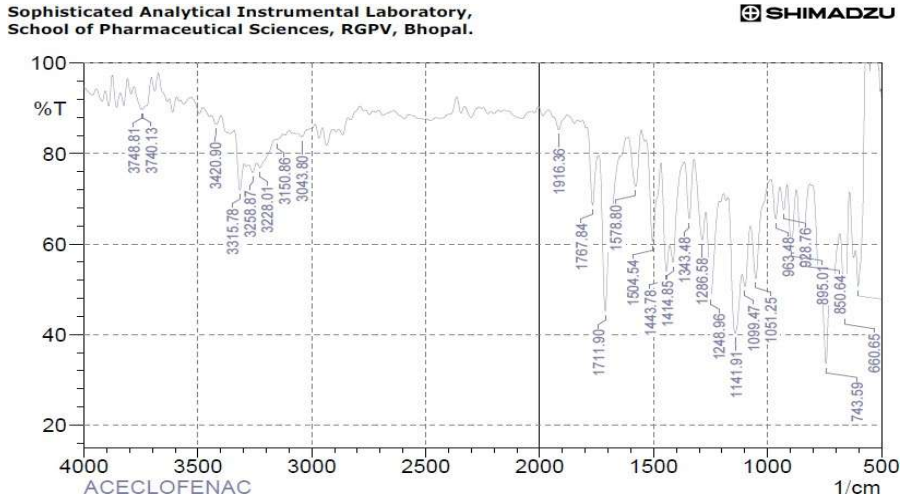

Fig 3: FTIR spectrum of Aceclofenac

Table 8: FTIR Interpretation data of Aceclofenac

S. No.	FTIR Peak (cm^{-1})	Interference
1.	3315.78	N-H str.
2.	3420.90	O-H str.
3.	1711.90	C=O str.
4.	1443.78	C-C str.
5.	743.59	C-Cl

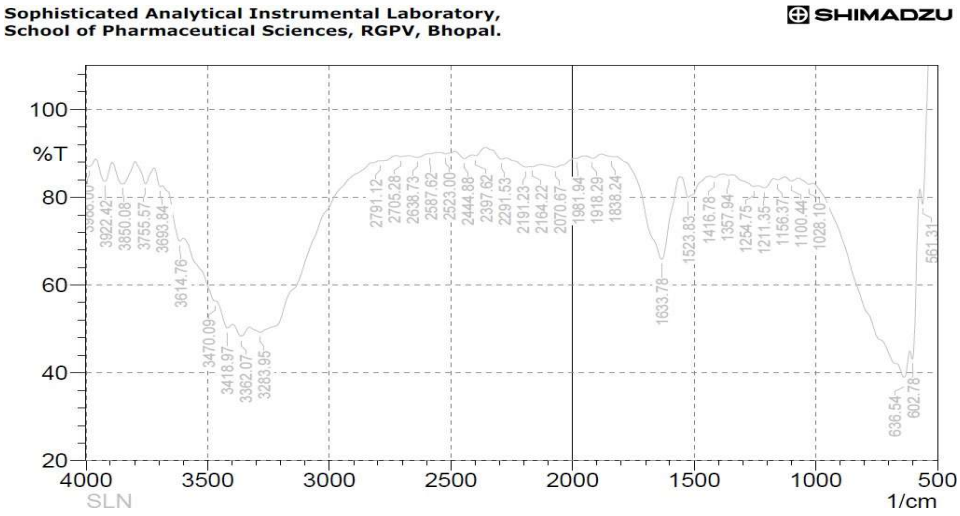

Fig 4: FTIR spectrum of Solid Lipid Nanoparticle Formulation

Table 9: FTIR Interpretation data of Solid Lipid Nanoparticle Formulation

S.No.	FTIR Peak (cm^{-1})	Interference
1.	3362.07	N-H
2.	636.54	C-Cl
3.	1633.78	C=O
4.	3418.97	O-H
5.	1523.83	C=C

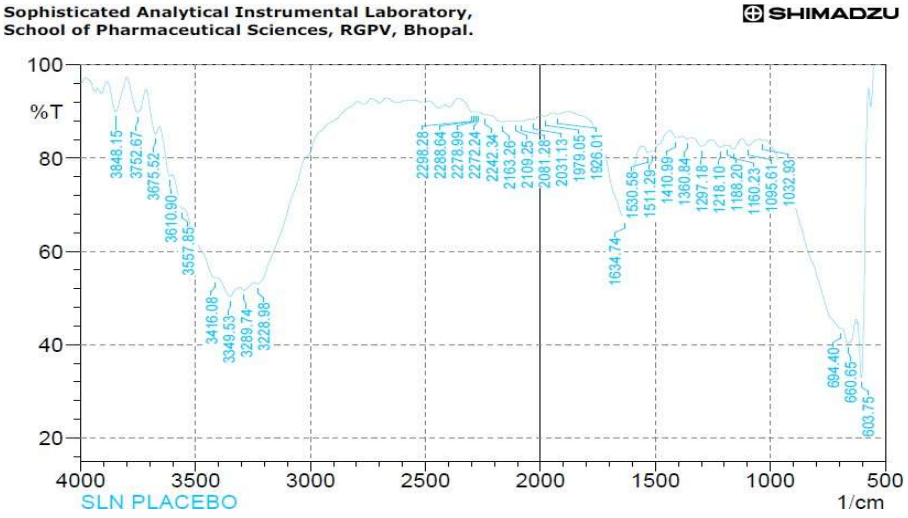

Fig. 5: FTIR spectrum of Solid Lipid Nanoparticle Placebo

Table 10: FTIR Interpretation data of Solid Lipid Nanoparticle Placebo

S.No.	FTIR Peak (cm^{-1})	Interference
1.	3416.08	O-H
2.	1530.58	C=C
3.	1634.74	C=O
4.	3228.98	C-H

Solid Lipid Nanoparticle Evaluation Parameter

Solid Lipid Nanoparticle Evaluation Parameter Particle size, P.I., Zeta potential, Entrapment efficiency (% E.E.), show in table no 11

Table 11: Particle size, P.I., Zeta potential, Entrapment efficiency (% E.E.)
"

Run	Factor 1 A: Lipid	Factor 2 B: Surfactant	Factor 3 C: Extract	mean particle size	PDI	Zeta potential	Drug Entrapment Efficiency (Ace)	Drug Entrapment Efficiency (Pip)
Batch	**mg**	**µl**	**mg**	**nm**	**Unit less**	**mV**	**(%)**	**(%)**
1	200	600	200	98.1	0.427	-2.7	68.73	65.55
2	300	600	200	112.8	0.423	31.5	71.97	75.54
3	200	700	250	94.7	0.436	-2.1	74.56	76.14
4	250	600	250	115.7	0.397	-11.7	67.43	62.09
5	250	700	200	103.3	0.418	-14.5	69.54	66.18
6	250	600	250	111	0.405	-8	72.17	64.19
7	300	500	250	96.5	0.356	34.4	76.23	83.86
8	250	700	300	104.8	0.405	-10.8	77.55	81.98
9	250	500	200	98.3	0.399	-7.9	74.15	75.36
10	250	600	250	91.5	0.435	-11.7	78.9	82.84
11	200	500	250	82.8	0.384	80.2	83.27	82.3
12	250	600	250	92.3	0.439	-8	84.25	88.31
13	250	600	250	89.2	0.439	-8	83.51	83.89
14	250	500	300	127.4	0.361	-96.4	84.51	81.78
15	200	600	300	92.9	0.373	32.2	76.48	79.11
16	300	700	250	96.8	0.386	1.9	77.51	70.49
17	300	600	300	95.8	0.303	74	80.75	76.47

In-vitro % drug release of Aceclofenac And Piperine for 1 hr, 2 hr, 3 hr, 5 hr, 24 hr shown in table no.12

Table 12: In-vitro % drug release of Aceclofenac And Piperine

Run	Factor 1 A: Lipid	Factor 2 B: Surfactant	Factor 3 C: Extract	. *In-vitro %* drug release									
				1 hr (Ace)	1 hr (Pip)	2 hr (Ace)	2 hr (Pip)	3 hr (Ace)	3 hr (Pip)	5 hr (Ace)	5 hr (Pip)	24 hr (Ace)	24 hr (Pip)
Batch	**mg**	**µl**	**mg**	**(%)**	**(%)**	**(%)**	**(%)**	**(%)**	**(%)**	**(%)**	**(%)**	**(%)**	**(%)**
1	200	600	200	8.8	9.28	19.14	14.56	35.2	20.37	46.86	29.93	87.42	59.19
2	300	600	200	8.628	9.74	19.71	14.23	24.28	20.2	44.86	31.19	80	57.69
3	200	700	250	9.31	9.97	13.37	14.21	21.88	20.35	32.57	30.54	84.05	58.61
4	250	600	250	10.05	9.97	15.02	13.24	22.85	18.7	38.85	28.81	88	57.58
5	250	700	200	9.2	9.78	17.31	13.04	26.34	17.92	38	27.65	86.22	55.63
6	250	600	250	8.4	8.96	19.37	12.07	28.34	17.15	38.8	24.93	89.08	55.44
7	300	500	250	7.94	6.97	13.54	10.63	23.77	15.98	38.97	22.98	83.82	55.24
8	250	700	300	7.88	7.83	13.65	12.26	20.57	16.95	38.45	22.4	87.71	58.74
9	250	500	200	8.28	5.11	13.54	9.15	22.8	14.23	41.71	23.17	86.11	54.62
10	250	600	250	7.54	7.03	15.14	10.71	27.37	14.62	40.17	20.65	89.71	55.63
11	200	500	250	8.8	6.08	14.68	9.35	22	14.03	35.2	24.32	88.97	55.38
12	250	600	250	8.97	6.28	16.57	9.17	25.42	12.86	40.85	21.04	88.91	54.47
13	250	600	250	7.94	6.47	14.97	10.51	25.42	14.56	40.57	22.98	85.65	54.82

14	250	500	300	8.34	6.35	16.11	9.73	28.34	14.62	42.68	22.49	89.71	55.24
15	200	600	300	7.82	8.41	13.37	12.46	22.11	14.01	39.48	24.34	86.28	58.36
16	300	700	250	9.42	6	15.25	10.12	22.45	14.52	40.34	22.81	89.31	56.04
17	300	600	300	8.07	6.2	12.57	10.44	20.62	14.54	39.94	23	88.4	57.19

***In-vivo* Skin Irritation Study**

The skin of all animals in the treatment group was applied with Carbopol gel base and Carbopol SLN gel formulation and was observed at 30 minutes, 24 hours, 48 hours, and 72 hours and was found completely normal and showed no visible abnormality.

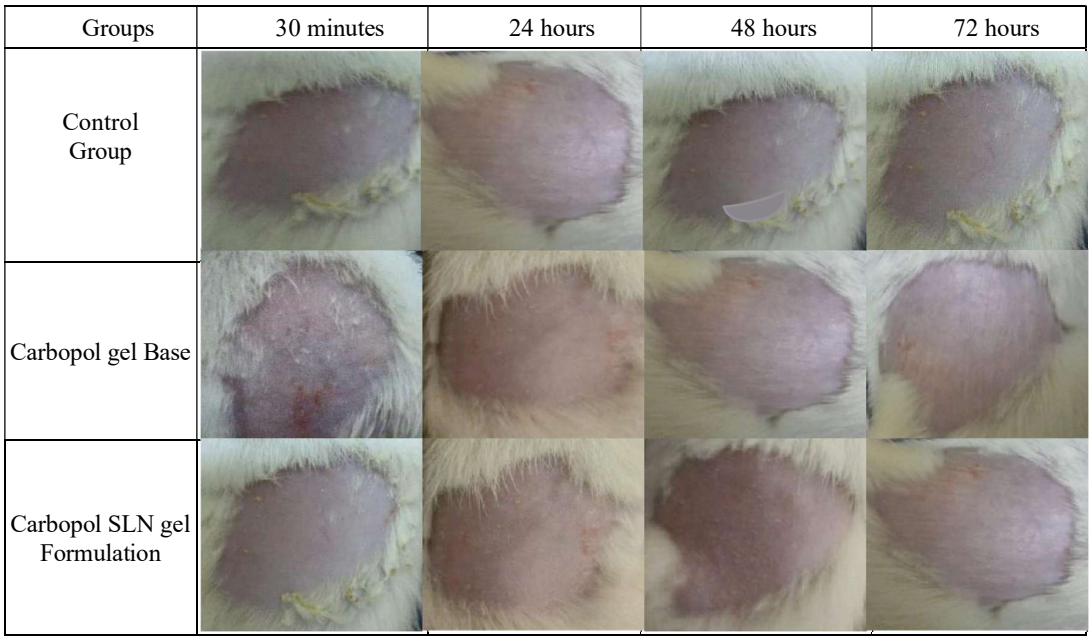

Fig 6: Skin irritation from 30 mins of application to 72 hours of application of the formulation.

Table 13: Skin irritation Observation

S. No.	Observations	Score
1.	30 minutes	**0**
2.	24 hours	**0**
3.	48 hours	**0**
4.	72 hours	**0**

DISCUSSIONS

Formulation of Solid Lipid Nanoparticles

Since Aceclofenac is a BCS Class- II drug, and Piperine may cause irritation if directly applied on to the skin, so they were entrapped in solid lipids by ultra-sonication method. High speed of stirring results in better drug loading and decreased particle size.

Optimization of Aceclofenac and Piperine Loaded SLNs

The BBD experimental design was used because of its advantage of requiring fewer runs than other designs, which might require more runs. The mean PS, PDI, ZP, drug EE%, and drug release at various time periods are well recognized CQAs of drug loaded SLNs; therefore they are selected as response variables. The relationship between each independent variable and response variable and effect of interaction of multiple independent variables at varied levels on each response variable of SLN formulation was individually assessed.

CONCLUSION

The Aceclofenac and Piperine loaded SLN formulation was systematically optimized by Box- Behnken design approach using Design Expert software. A three-factor, three-level BBD scientifically explained the relationship between selected independent variables and targeted response variables with designing of only 17 optimization trial batches.

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