



FORMULATION AND EVALUATION OF SOLID LIPID NANOPARTICLE (SLN) BASED TOPICAL GEL OF IBUPROFEN

Parul Raje* and Shikha Agrawal

Department of Pharmaceutics, Swami Vivekanand College of Pharmacy, Indore 452020,
Madhya Pradesh, India.

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*Corresponding Author

Parul Raje

Department of
Pharmaceutics, Swami
Vivekanand College of
Pharmacy, Indore 452020,
Madhya Pradesh, India.

ABSTRACT

Objective: The aim of the present investigation is to formulate & evaluate the solid lipid nanoparticles (SLN) based topical gel of ibuprofen for the treatment of mild to moderate pain, which would avoid the gastrointestinal-related toxicities associated with oral administration. **Methods:** Solid lipid nanoparticles were formulated by melt emulsification and solidification at low-temperature method using stearic acid & tween 80. Various batches of SLN were prepared using different concentrations of lipid and surfactant, then SLN were incorporated into carbopol gel base to form a gel. **Results:** All the Preformulation parameters of the drug were evaluated such as physical characterization, melting point, pH, identification by UV spectroscopy

and FTIR analytical method, preparation of calibration curves, solubility. Then SLN were subjected to scanning electron microscopy, drug entrapment efficiency (EE), particle size determination. It has been observed that high lipid concentration containing formulation have higher entrapment as. Finally, the formulation of SLN based topical gel was subjected to further study such as physical appearance, pH, viscosity determined using a Brookfield and Spreadability. The In-Vitro drug release of all the formulated gel was evaluated using Modified Franz diffusion cell containing dialysis membrane and phosphate buffer having pH 7.4 in the receptor medium. The in-vitro release was carried out in comparison with a marketed gel (Ribufen® gel). **Conclusion:** The research work concluded that all the Ibuprofen loaded SLN based topical gel formulation containing carbopol was suitable for topical application and having good penetration power.

KEYWORDS: SLN, Ibuprofen, carbopol, Topical Gel.

INTRODUCTION

Ibuprofen [2-(4-isobutylphenyl) propionic acid] is a potent non-steroidal anti-inflammatory (NSAID) drug which is used for the treatment of pain caused by acute and chronic arthritic condition and other. Ibuprofen is also used in primarily to treat fever (including post immunization fever), mild to moderate pain (including pain relief after surgery), painful menstruation, osteoarthritis, dental pain, headaches, and pain occurs by kidney stones. At the time of absorption, due to the poor water solubility of ibuprofen drug in oral forms results in low bioavailability and incomplete absorption from the gastrointestinal tract. In addition to absorption difficulties, oral formulations of ibuprofen can cause harm to gastric mucosa, which may result in ulceration and bleeding. The current approaches aim to decrease ibuprofen related adverse effects caused by oral administration and also improve the penetration power using SLN.^[1]

The Colloidal particles having range in size between 10 and 1000 nm are known as nanoparticles. Nanoparticles are manufactured from both the synthetic or natural polymers and ideally suitable to reduce toxicity. Over the years, they have emerged as a variable substitute to liposomes as drug carriers. The formulation of SLN for drug delivery depends on their ability to penetrate through several anatomical barriers, sustained release of their contents and their stability in the nanometer size.^[2] The Solid Lipid Nanoparticles (SLN) are alternative drug carrier systems. Solid lipid nanoparticles composed of solid lipids and having size ranges between 100 – 1000 nm. In the past few years, lipid matrices became extremely popular in controlling the release of drugs. *In-vitro* drug release pattern of SLN based gel showed fast and control release. The immediate release can be useful to improve the penetration of drug, while sustained-release supplied the drug over a prolonged period of time.^[3,4]

MATERIALS AND METHODS

Material

All the chemicals including Drug (Ibuprofen) were received from S.D. Fine Chem Ltd., Mumbai. Dialysis membrane was purchased from Hi-Media Laboratories Pvt. Ltd., Mumbai. All the reagents and solvents were of analytical reagent (AR) grade.

Methods

Preformulation studies

The physicochemical parameters of drug substance cover the pre-formulation studies, which are characterized with the goal of designing optimum drug delivery system.

Organoleptic properties

The pure drug sample was studied for their organoleptic properties like color, odor, taste, and crystallinity.

Melting point

In this method, a small amount of drug was filled in a capillary tube open both the ends and it was placed along with thermometer in melting point apparatus (BTI-34 melting point apparatus, Mumbai, India).

Solubility Analysis

Preformulation solubility analysis was done, which include the selection of suitable solvent, to dissolve the respective drug. The solubility study was done by adding the small amounts of solute to the fixed volume of solvents, after each addition, the system was vigorously Shaken and examined visually for the undissolved solute particles.

Partition coefficient

The partition coefficient study of ibuprofen was performed using n-octanol as the oil phase and water (1:1) as the aqueous phase. The two phases were mixed in equal quantities (50 ml) by adding 50 mg of drug in a separating funnel and was saturated with each other at room temperature for 24 hr. The saturated phases were separated by centrifugation. The two phases were separated and then analyzed for respective drug contents.^[5] The partition coefficient measured by the given formula:

$$K(o/w) = \frac{\text{conc. of the drug in oil phase}}{\text{conc. of the drug in an aqueous phase}}$$

Where,

K = Partition coefficient

Spectrophotometric analysis

Preparation of calibration curve of ibuprofen in sodium hydroxide (λ_{max} 254 nm)

A standard stock solution of ibuprofen was prepared by dissolving 100 mg of drug in 100 ml of 0.1M NaOH (1000 $\mu\text{g/ml}$). From the above stock solution, 10 ml was taken and diluted up to 100 ml in NaOH (100 $\mu\text{g/ml}$). From the above solution 1, 2, 3, 4, 5 and 6 ml was taken and diluted up to 10 ml with NaOH to get series of solutions in a concentration range from 10 to 60 $\mu\text{g/ml}$ of ibuprofen. Absorbance was noted using UV-VIS Spectrophotometer at λ_{max} of 254 nm against a blank (NaOH solution).^[6]

FTIR study

The IR analysis of the sample was carried out for qualitative identification. In ATR (Attenuated Total Reflectance), the solid material was placed onto the small crystal area. In this instrument IR Affinity-1, (Shimadzu, Japan) diamond being the preferred choice for most applications because of its robustness and durability. After the solid sample has been placed on the crystal area, the pressure arm was arranged as over the crystal/sample area. Then force was applied to the sample by pushing it onto the diamond surface. Transmittance was measured from wave number 4000 cm^{-1} to 400-1 using Happ-Gensel apodization.

Preparation of Ibuprofen loaded SLN

SLN loaded ibuprofen drug were prepared using melt emulsification and low-temperature Solidification method. Ibuprofen was dissolved in methanol and mixed with the acetone solution containing stearic acid (Lipid). The mixtures were sonicated for 15 minutes, and then added dropwise to Tween 80 (Surfactant) solution, the mixture was stirred at 3000 rpm for 30 min at 70 °C temperature. The mixed solution was transferred to ice water bath and stirring for 4 hours at 3000 rpm. Different formulations of drug loaded SLN (S1, S2, S3, S4, S5, and S6) were prepared by varying concentrations of stearic acid and surfactant.^[5] Composition for SLN formulation shown in Table -1.

Table 1: Composition of different SLN.

Formulations	Drug (mg)	Stearic Acid (mg)	Tween 80 (%)
S1	300	1000	2.5
S2	300	1250	2.5
S3	300	1500	2.5
S4	300	1000	2.0
S5	300	1250	2.0
S6	300	1500	2.0

Characterization of Ibuprofen Loaded SLN

The characterization parameter of SLN like Particle size and size distribution, zeta potential, drug entrapment efficiency (EE), scanning electron microscopy (SEM) were determined.

Scanning Electron Microscopy

The morphological characteristic of SLN was determined by scanning electron microscope (JEOL-JSM-6360 Japan). One drop of sample was placed on a slide and excess water was left to dry at room temperature.^[7] then the slide was attached to the specimen holder using a double-coated adhesive tape and gold coated under vacuum using a sputter coater (Model JFC-1100, Jeol, Japan) for 10 minutes and investigated at 20kV.^[8]

Drug entrapment efficiency (EE)

The entrapment efficiency of SLN loaded ibuprofen was calculated by using 100 mg of SLN dissolved in the dispersion medium and the solution was centrifuged at 12000 rpm. The supernatant fluid was collected and passed through a membrane filter. The quantity of drug in the solution was measured by UV spectroscopy at 263nm.^[9]

$$\% \text{ EE} = \frac{W1 \text{ initial drug} - W2 \text{ free drug}}{W1 \text{ initial drug}} \times 100$$

Where EE is entrapment efficiency, W1 stands for the mass of ibuprofen added to the formulation and W2 is the analyzed weight of drug in the supernatant layer.^[10]

Particle size, Zeta potential & Particle Size Distribution

The mean particle size and polydispersity index of SLN for size distribution was measured using Malvern Mastersizer 2000MU (Malvern instrument the UK). The obtained data were calculated using the volume distribution (D10%, D50%, D90 %).^[11]

The PI was measured by the SPAN which can be calculated from the following equation.

$$\text{SPAN} = \frac{D 90\% - D 10\%}{D 50\%}$$

Where,

D90% - particle diameter at 90% cumulative size,

D10% - particle diameter at 10% cumulative size,

D50% - particle diameter at 50% cumulative size.

Zeta potential of SLNs was measured by using Zetasizer 2000 (Malvern Instruments, UK) at 25°C.^[12-15]

Formulation of Ibuprofen Loaded SLN Based Gel

SLN loaded gel was prepared by using required quantity of carbopol 934 (gelling agent). In this method required amount of carbopol was weighed and dissolved in small volume of distilled water to prepare aqueous dispersion and the dispersed carbopol was allowed to hydrate for 4 to 5 hour. Glycerin (10% w/w) used as humectants was added subsequently to the aqueous dispersion equivalent to 10% of ibuprofen was incorporated in it.^[16] Triethanolamine (viscosity modifier), was added to the carbopol dispersion using stirrer at 1200 rpm. Stirring was continued till the carbopol get dispersed. Then the formulated gel was allowed to stand overnight for the removal of entrapped air. Different formulations of gel (G1, G2, G3, G4, G5 and G6) using different batches of SLN were prepared. Then gel was allowed to further investigations such physical appearance, pH, viscosity, spreadability and in-vitro drug release. Composition for SLN based gel formulation shown in Table -2.^[17]

Table 2: Composition of SLN Gel formulation.

Ingredients	Composition (%W/W)
Carbopol 934	1
SLN eq. to 10% of ibuprofen	1
Glycerin	10
Triethanolamine	q.s
Distilled water	q.s

Evaluation methods of ibuprofen –loaded SLN gel

Physical Evaluation

Physical appearance such as color and appearance were observed.^[18]

Determination of Viscosity, pH

Viscosity of the formulated gels was determined using a Brookfield Viscometer using Spindle type 93/T-C.^[19]

1gm of gel was taken and dissolved in 100ml of purified water and stored for two hour. The three samples were checked for pH using pH meter.^[20]

Determination of Spreadability

Two sets of glass slides of standard dimensions were taken. The gel formulation was placed over one of the slides.^[21] The other slide was placed on the top of the gel, 100gm weight was placed upon the upper slides so that the gel between the two slides was pressed uniformly to form a thin layer. A 20 gm weight was tied to the upper slide carefully. The time taken for the

upper slide to travel the distance of 6.0 cm and separated away from the lower slide under the influence of the weight was noted. The experiment was repeated by three times and the mean time taken for calculation.^[22]

Spreadability was calculated by using the following formula:

$$S = m \times l / t$$

Where,

S - Spreadability

m - Weight tied to the upper slide (20gm)

l - Length of the glass (6 cm)

t - Time taken in seconds

In-vitro drug release studies of SLN Based Gel

The *In- Vitro* release studies were performed using modified Franz diffusion cell to evaluate the amount of ibuprofen released from each formulation. These cells consist of donor compartment, acceptor compartment, Dialysis membrane 70, magnetic stirring, thermostatic water bath and sampling device.^[23]



Fig. 1: Modified Franz Diffusion Cell.

Dialysis membrane 70 (Hi-Media, Mumbai, India) having pore size 2.4 nm, molecular weight cut-off between 12,000-14,000 was used and mounted on the Franz diffusion cells.^[24] The surface area of the release membrane was 3.14 cm². The receptor medium was approximately 50 ml and composed of phosphate buffer saline (PBS), pH 7.4, and stirred by the magnetic bar at 700 rpm to avoid different concentrations within the acceptor medium and to minimize stagnant layers.^[25-26] SLN Based gels (equivalent to 30 mg of drug) were placed in the donor compartment. During the experiments, the solution in receptor side was maintained at 37°C ± 0.5°C.^[27-29] After a certain time interval, 2 ml of the sample was withdrawn at different time

intervals from receiver compartment through side tube and same volumes of freshly prepared receptor medium were added. The samples were analyzed by UV-VISIBLE spectrophotometer at 263nm. For each formulation, the release studies were performed in triplicate and mean was taken.^[30] Franz cell glass structure represented in given Fig.1.

RESULTS AND DISCUSSION

Estimation of ibuprofen: The calibration curve of drug obeyed Beer Lambert's law in the concentration range of 0-50 µg/ml ($R^2 = 0.999$) and result shown in fig- 2

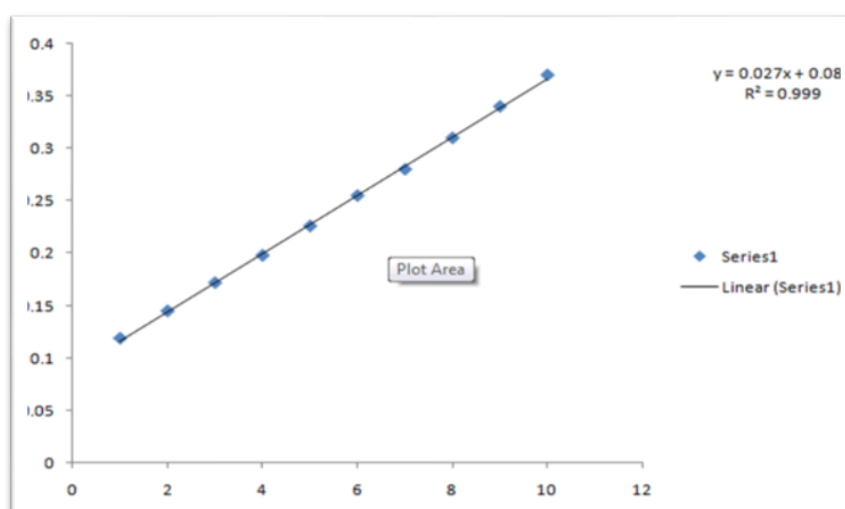


Fig. 2: Calibration curve of ibuprofen at λ_{\max} -254nm.

Table 3: Physiochemical parameters.

Parameters	Results
Organoleptic Properties	White crystalline, powder, Odourless.
Partition Coefficient	4.6
Melting Point	76 °C

Solubility Analysis results

The drug was subjected to solubility analysis and the result obtained are given in Table – 4.

Table – 4 Solubility determination.

Solvents	Results
Acetone	+++
Chloroform	+++
Methanol	+++
Distilled water	-
PBS 7.4	++

[Insoluble –, Soluble ++, Freely soluble +++]

Compatibility study by FTIR

The FTIR spectra of pure ibuprofen and physical mixture of the drug with stearic acid (1:1). The IR spectra of the pure drug show principle peaks at 1721 cm^{-1} (C=O stretching Vibrations of -COOH group), $870, 779\text{ cm}^{-1}$ (Aromatic stretching bending vibration).

The physical mixture shows peaks at $1720.72, 865.51$. Thus it concluded that the physical mixture of the drug ibuprofen does not show any major interactions with formulation components like lipid (Stearic acid). The result is shown in Fig.3 and Fig. 4.

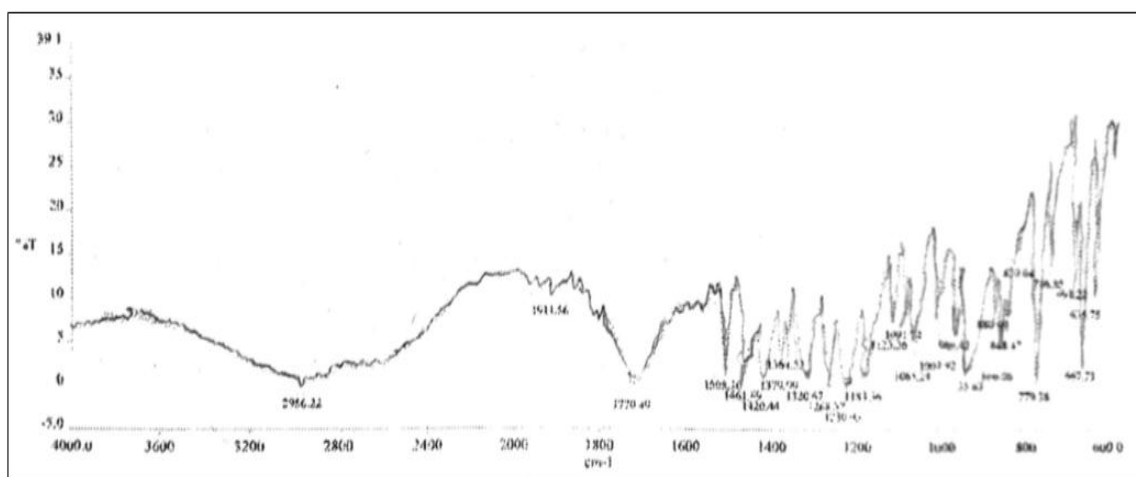


Fig. 3: FTIR Spectra of pure ibuprofen.

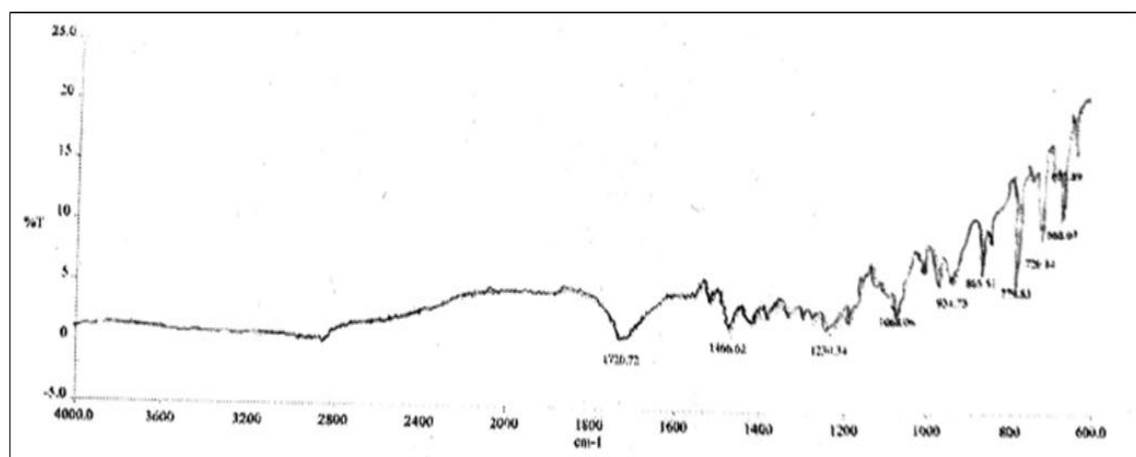


Fig. 4: FTIR Spectra of a physical mixture of ibuprofen and stearic acid.

Evaluation of SLN loaded gel.

Physical evaluation of Gel shows white color and Translucent appearance.

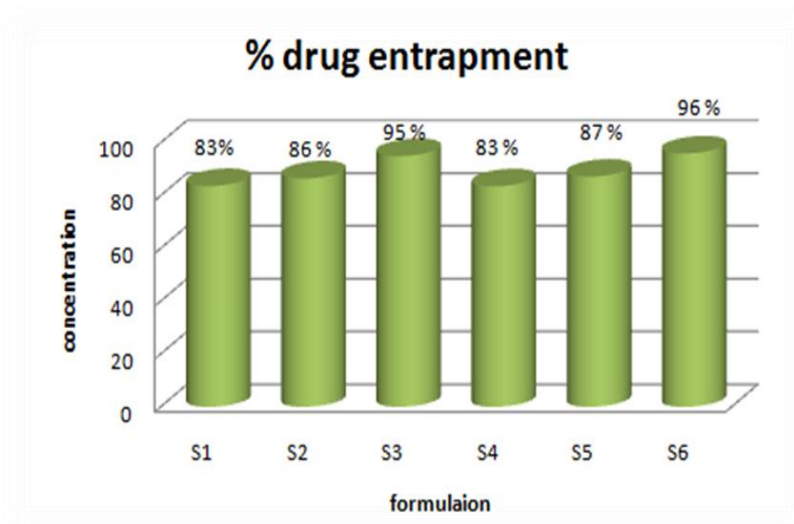
Table 5: pH, Viscosity & Spreadability.

Formulation	pH	Viscosity (Cps)	Spreadability (gm-cm/sec)
G1	6.8	2290.24±0.25	2.80±0.80
G2	7.2	2480.26±0.22	3.20±0.40
G3	7.2	2660.22±0.24	3.45±0.61

pH, Viscosity, and Spreadability values were found out to be complacent as a skin preparation. Spreadability of the preparation was also found out to be satisfying and assures the suitability. The results show good spreadability and homogeneity. The pH of the gel formulations is within range of 6.8 to 7.2, which lies in the normal pH range of the skin and would not produce any irritation to the skin. The viscosity of gel also within the range of 2591.26cps to 3127.66cps and spreadability in range of 3.65 to 3.22 gm-cm/sec as result shown in Table-5.

Percent Entrapment efficiency

The Solid lipid nanoparticles were prepared by different proportions of Drug, Stearic acid, and surfactant. The entrapment efficiency of Solid lipid nanoparticle loaded ibuprofen increases with increase in the concentration of stearic acid and Tween 80. The entrapment efficiency found in the range between 83.55%-96.00%. Formulation (S3 and S6) of SLN has shown maximum entrapment (that is 95.00% 96.00%) as the concentration of lipid increases, while Formulation (S1 and S4) of SLN has shown lowest entrapment that is (83.66% and 83.55%) as the concentration of lipid decreases as result shown in Fig. 5.

**Fig. 5: Percent drug entrapment.**

Scanning Electron Microscopy

Surface analysis of Solid Lipid nanoparticle was carried out by Scanning Electron Microscopy, Images obtained after SEM are shown in Figures, SLN 1 and SLN 3 and SLN 6. SLN 1 have quite a rough surface, SLN 3 are almost spherical in shape. SLN 1 and SLN 3 have a smaller size as compare to SLN 6 because of higher concentration of Tween in SLN 1 and SLN 3. SLM 6 have spherical- shape, and fine- smooth surface. Almost all of them are spherical in shape, and have fine, smooth surface as results show in Fig. 6, Fig. 7 and Fig. 8.

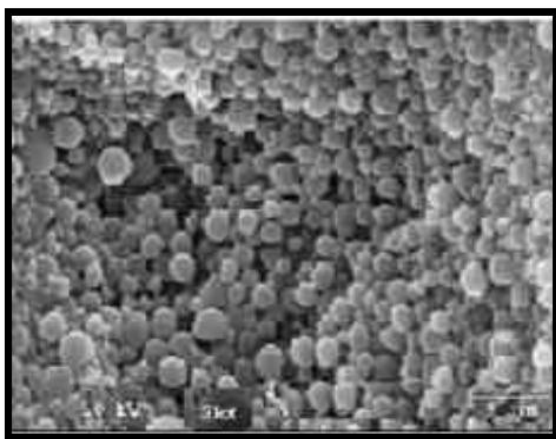


Fig. 6: SLN1

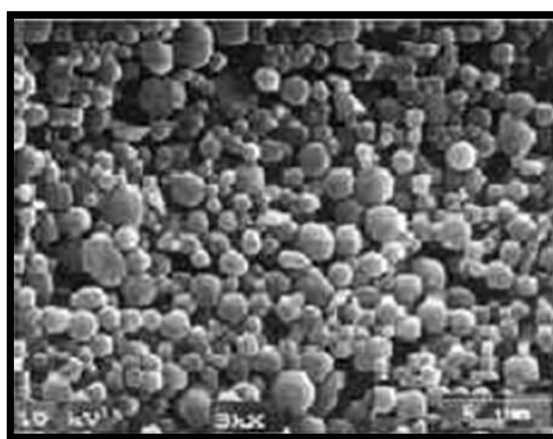


Fig. 7: SLN 3.

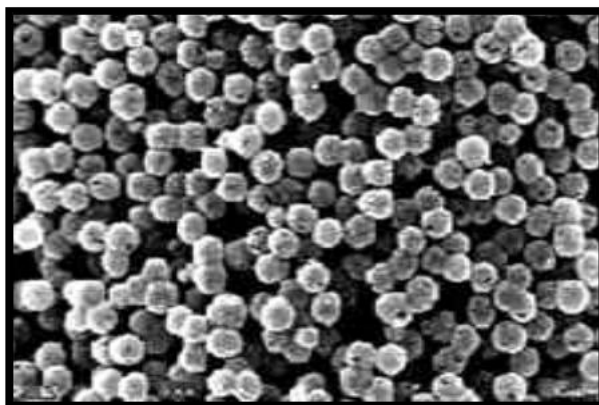


Fig. 8: SLN 6.

Particle size, Zeta potential & Particle size distribution

The D90% for SLN F1, F2, F3, F4, F5, F6 and Blank SLN determined using Malvern Mastersizer showed size 1527, 2241, 334, 7232, 1286 and 4264 nm respectively. The particle sizes of formulations, increases as the concentration of tween 80 decreases. Zeta potential of SLN 3 was found -25.2 and obtained results shown in Table- 6, Fig. 9 and Fig.10.

Table 6: Particle size, Zeta potential & Particle size distribution.

Formulation code	Mean volume distribution			Span	Zeta potential of SLN Dispersion(mV)
	D10%	D50%	D90%		
SLN-F1	0.125	0.176	1.527	6.983	-22.8
SLN-F2	0.126	0.172	2.241	1.673	-24.2
SLN-F3	0.128	0.173	1.334	1.633	-25.5
SLN-F4	0.148	0.326	7.432	20.292	-20.4
SLN-F5	0.146	0.204	1.286	6.752	-20.6
SLN-F6	0.124	0.326	2.264	10.771	-21.5

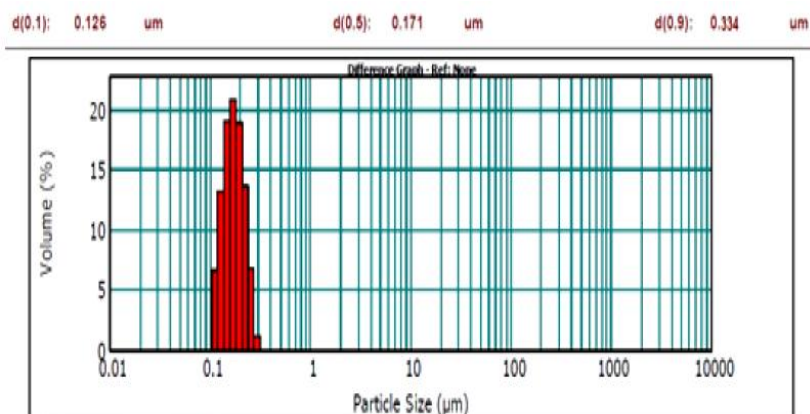


Fig 9: Particle Size distribution Graph (SLN 3).

RESULTS	Mean(mV)	Area (%)	Width (mV)
Zeta potential (mV)	Peak 1 -25.6	98.6	6.00
Zeta Deviation (mV)	Peak 2 -52.6	0.2	0.01
Conductivity (mS/cm)	Peak 3 0.00	0.0	0.00
Result quality : Good			

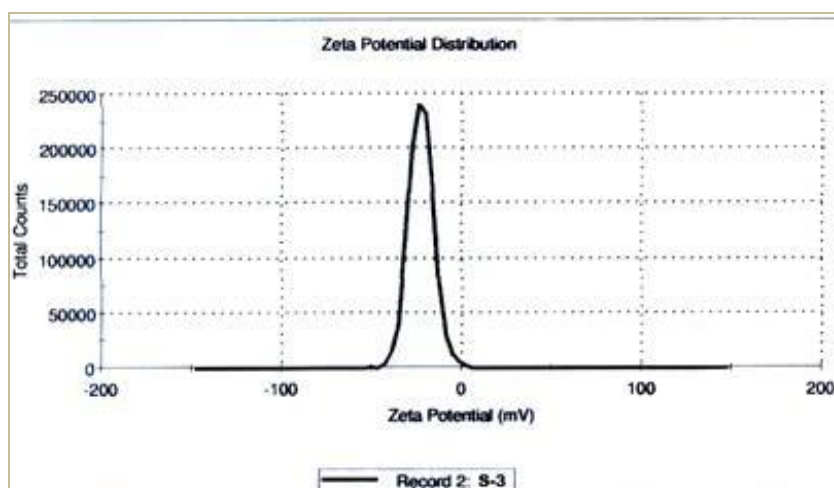


Fig 10: Particle Size distribution Graph (SLN 3).

Comparative In-vitro drug release studies of formulated gel with marketed gel

Drug diffusion study was performed on all formulations G1, G2, G3, G4, G5 and G6 using Franz diffusion cell. All the formulation were compared with marketed gel (Ribufen). The cumulative % release of gel from formulations F1 to F6 have good penetration power, % drug release and prolong effect as compared to marketed preparation (Ribufen). The release of the gel was then sustained over a period of 360 min. The results of this study signify that administration of gel in nanoparticle carrier is safer compared to administration of the free drug and oral formulations. The obtained results are shown in Table- 7 and Fig. 11.

Table 7: Comparative In-vitro release studies:

Time	G1	G2	G3	G4	G5	G6	Ribufen®
0	0.11	0.12	0.14	0.11	0.13	0.18	0.11
30	1.41	1.56	1.64	1.36	1.58	1.72	1.13
60	2.21	2.66	3.12	2.22	2.82	3.22	3.12
120	12.12	12.23	13.11	13.66	13.24	14.18	14.13
180	25.82	25.77	28.12	26.22	27.44	28.44	23.66
240	52.61	58.92	60.16	56.12	57.64	59.19	51.44
300	70.22	71.61	76.55	78.18	79.84	82.18	65.64
360	83.44	88.12	90.14	92.33	94.19	96.32	78.45

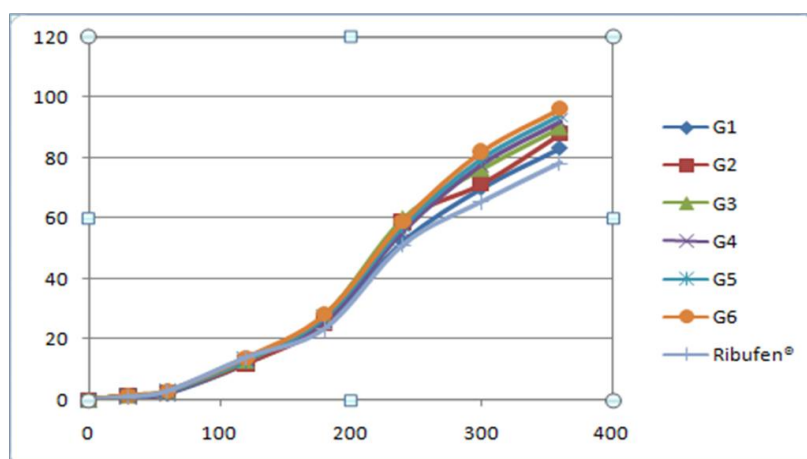


Fig. 11: In- vitro drug release.

CONCLUSION

Solid lipid nanoparticles were prepared by melt emulsification and low-temperature Solidification method, which was found to have simple and economic. Ingredients used in this study were economic and safe. Characterization of SLN reveals a good kind of product which could be reproduced for commercial purpose. Entrapment efficiency, drug release and viscosity were good and up to the acceptable range.

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CONFLICT OF INTERESTS

The authors declare that they have no conflict of interest.

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