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Development and Evaluation of Solid Lipid Nanoparticles Loaded Gel of Berberine for Topical Drug Delivery

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ABSTRACT
Berberine alkaloids belong to a group of naturally occurring chemical compounds that mostly contain basic nitrogen atoms. Berberine is iso-quinoline derivatives and belong to protoberberine. Berberine is a yellow plant extract with quaternary ammonium salt of an iso-quinoline alkaloid and active component of various Chinese herbs, with potential antineoplastic, radio-sensitizing, anti-inflammatory, anti-lipidemic and antidiabetic activities.
The aim of the present research work was to develop and optimize Solid Lipid Nanoparticles (SLNs) based gel of berberine and to evaluate their potential as topical drug delivery system for safe and effective management.
The solid lipid nanoparticulate gel was prepared using high-pressure homogenization technique and was incorporated into polymeric gels for convenient application. The nanoparticulate its gels were evaluated and characterized by various parameters including particle size, in vitro drug release.
The present study concludes that Solid Lipid Nanoparticles (SLNs) based gel of berberine is a safe and effective drug delivery system with improved bioavailability.
Keywords: Berberine; Solid lipid nanoparticles; gel; in-vitro release.

I. INTRODUCTION:

Solid Lipid Nanoparticles:

Another category of colloidal drug delivery vehicles known as SLNs are solid lipid-based molecules that are diffused in a diameter ranging of 10 to 1000 nm. Since they include the benefits of these colloidal systems yet avoiding their drawbacks, SLNs it was first designed as an alternative to microparticles as well as liposomes there in early 1990s [Mukherjee et al 2009]. [1-3] Optimal biomedical applications are SLNs' key strength over polymeric micelles as the lipids that are used in preparation are internal components of the body so are well tolerated, while their increased stability makes them superior to liposomes. Due to their favorable characters, Utilization of SLNs in I.V., I.M., oral, intestinal, ocular, cutaneous, and other modes of therapy have been demonstrated. [Naseri et al., 2015; Puri et al., 2009].

Components of SLN Formulations

Solid lipids and stabilizers are the main elements of SLNs. That lipid substrate with which the medicine is incorporated can consist of glycerol (such as tristearin and tripalmitin), partially or complete glycerides (such as glyceryl monostearate, glyceryl palmitostearate, and glyceryl monooleate), [4,5] steroid (such as lipid), or hydrocarbons (such as cetyl palmitate) (Scheffel 1970).

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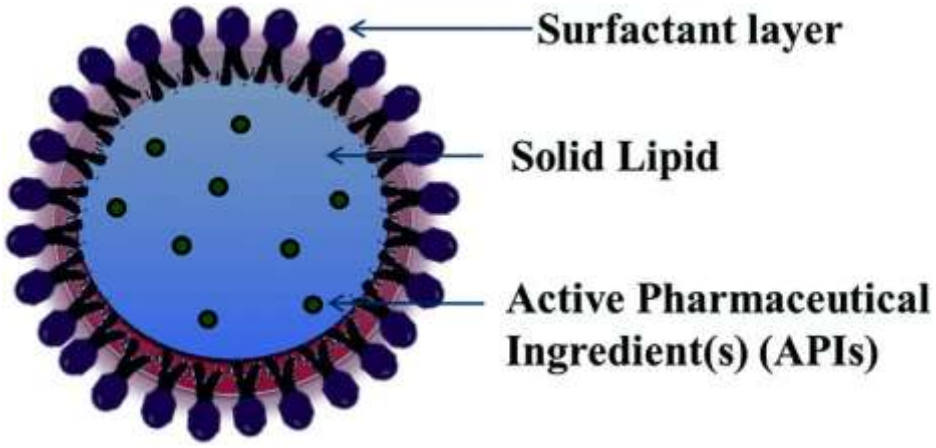

Fig.1: Solid Lipid Nanoparticles

II. MATERIALS AND METHODS:

Materials:

Berberine was received as a gift sample from Dr. Prashant Shukla, Associate Professor, Hygia Institute of Pharmaceutical Education and Research, Lucknow. All other chemicals sodium lauryl sulphate, tween 80, Triethanolamine were obtained from the laboratory of Hygia Institute of Pharmaceutical Education and Research, Lucknow. All the chemicals were analytical grade.

Methods:

BRB loaded SLNs Preparation

By heated homogenization proceeded mostly by ultrasonication procedure, berberine-loaded SLN got created. An ethanol, methanol, as well as acetone mixture was used to degrade the BRB and monoglyceride. Rotating flash evaporators were used to totally eliminate organic solvents. [6-8] Heating at 5°Cover the lipid's melting point caused the imbedded lipid layer to melt. The stabilizers (tween 80, poloxamer 188, or span 20) were dissolved in distilled water (enough to yield 30 ml) and heated to the exact same temperature as the oil phase to create the aqueous phase. The hot aqueous phase was introduced to the oil phase, also for 30 mins, homogenization being carried with a mechanical stirrer (at 2500 rpm & 70°C). The resulting gritty oil in water emulsion has been subjected to a 25-minute sonication process who used a probe Sonicator. The heated nano emulsion was ultimately allowed to cool to ambient temperature and then kept refrigerated at 4°C to produce BRB-loaded SLN. In Table 5.4, the various formulations' compositions are listed.

Formulation development and optimization of parameters:

In 5 ml of a water-miscible solvents at 70°C, the mono stearin got entirely dissolve. Three solvents—acetone, methanol, and ethanol—were utilized for screening. The creation of solid lipid nanoparticles was caused by injecting the resulting organic solvent into 50 ml of an aqueous phase that included solvent at a different temperature. The precise proportions of the materials added during processing were rigorously adhered observed.

Table 1: Batch formulation of SLNs

S.No.	Drug/Mg	Lipid gm	Surfactant	Rpm
1	16.6	33.3	750 mg	500
2	33.3	67.6	1000 mg	500
3	50	100	750 mg	750

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4	33.3	67.6	750 mg	750
5	50	100	1000 mg	750
6	33.3	67.3	750 mg	750
7	33.3	67.3	750 mg	750
8	50	100	750 mg	500
9	33.3	67.6	750 mg	750
10	16.6	33.3	750 mg	1000
11	16.6	33.3	1000 mg	750
12	50	100	500 mg	750
13	33.3	67.3	500 mg	500
14	16.33	33.3	500 mg	750
15	33.3	67.3	500 mg	1000
16	33.3	67.3	750 mg	750
17	33.3	67.3	1000 mg	12000

Fig.2: List of SLNs formulations

III. EVALUATION OF SOLID LIPID NANOPARTICLES: [9,10]

Evaluation SLNs loaded gel

Organoleptic evaluation

SLNs was visually evaluated for Organoleptic characteristics like general appearance, odor, and taste.

% Transmittance

% Transmittance of all the batches of a clove oil loaded microemulsion been 650 nm measurements

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were made with a UV-visible spectrophotometer while utilizing distilled water as a control.

Refractive index

RI of a SLNs has performed using a Abbe's refractometer by placing the sample in the sample cell and noting the reading of the refractometer.

pH of SLNs loaded gel

pH of 10% solution of SLNs loaded gel was observed using a digital pH meter. 10% solution of microemulsion based gel was prepared and the pH meter rod was inserted in the sample to record the pH of SLNs loaded gel. Findings were recorded in triplicate.

Viscosity

Applying a Brookfield RST cone plate rheometer, the permeability of a microemulsion-based gel as measured. In order to measure the viscosity of the formulation, On a plate, 2.0 g of gel has been put, the cone was fastened, and the sample spun axially between 10 and 100 RPM. Dependent on the sample's resistance toward rotation at each RPM, its sensor calculated the thickness of the material, and the results were displayed as the average of all the readings. The results were recorded in triplicate.

Drug Content

Using an UV - visible spectroscopy at the UV maximum of the relevant oil, the drug content of microemulsion-based gels of oils might have been assessed (Chen et al., 2004). And used the relevant essential oil as standards and the computation shown below, the amount of essential oil in the mixture was determined.[11]

Cu/Cs = Au/As

Whereas, Cs: conc of Reference drug, Cu: Con of sample, Au : absorbance of sample and As: absorbance of Refence drug.

Evaluation of entrapment efficiency (EE)

The EE of SLNs loaded with luliconazole estimated through described method with some modification. In brief, prepared SLN dried at room temperature then 5 10 ml of water contained 10 milligrammes of powdered SLN. HPLC grade ethanol and further proceeds by filtration through syringe filter of 0.22µm capacity. [12] Concentration of luliconazole determined spectrophotometrically at 299 nm. 52 (De Gaetano et al., 2021). The qualification taken in triplicate and based on percentage entrapment, best one selected for further evaluation. Entrapment efficiency has been determined according to following equation:

$$\%EE = \frac{Tdth - Sdth}{Tdth} \times 100$$

%EE – Percentage entrapment efficiency
Tdth – Total amount of DTH loaded to the product
Sdth – Amount of DTH found in supernatant
Tlipid – Total weight of lipid in the product

In-vitro drug release

The dialysis bag diffusion approach was used to assess the in-vitro discharge of BRB from various SLN dispersions. The medicine equivalent of 2.5 mg of Ramipril was introduced to a dialysis bag after being precisely weighed out in SLN dispersions. Following that, the wrapped bag has been placed in a beaker with 250 ml of phosphate saline pH 7.4 and swirled at a continuous 50 rpm speed at 37°C 0.5°C. At scheduled times, aliquots were removed from the graduated cylinder for up to 12 hours and replaced with new buffer (Ekambaram et al., 2011). The amount of medication released out from nanoparticles was then calculated spectrophotometrically using analyzing the absorbance at 207 nm employing the appropriate receptor media as a reference. [13]

Scanning electron microscopy and particle size determination

Photon correlation spectroscopy (PCS) was used to measure the mean diameter of the SLNs in the scattering at 25 °C and a stable angle of 90 ° [7]. One drops of sample was isolated per each chosen formed microemulsion prior to the measurement, and it was diluted in 10 ml of the dissolution medium (distilled water). SEM was used to describe the morphologies of SLN (Mohtar et al., 2015).

FTIR studies

FT-IR investigations were used to determine how the medication and lipids interacted. Studies on stability in order to discover any changes in the entrapment effectiveness of the SLN, stability experiments were conducted for the formulations with high entrapment capacity by maintaining the mixtures at two separate temperatures, 4°C and 25 2°C.

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Identification & characterization of Berberine (BRB)

FTIR Spectrometric study

The FTIR spectrum of the pure drug sample was recorded and interpreted in figure 3. Interpretation of the IR spectrum with corresponding functional groups. The spectrum was compared with the standard spectra. The obtained spectrum was matching with standard.

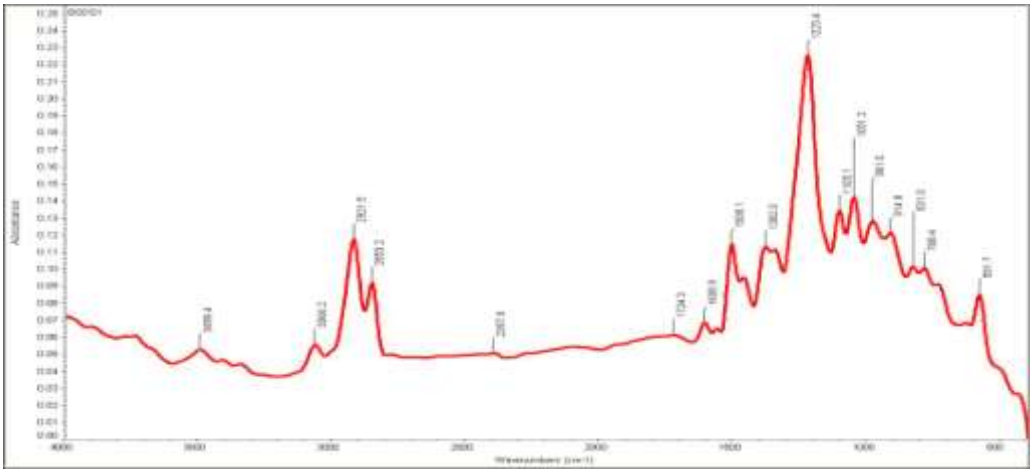

Fig 3: FTIR spectrum of BRB

DSC Analysis and Melting point

The capillary method was utilized to determine the BRB's boiling range, which ranged from 140 to 151°C. Further confirmation was done by DSC study. The DSC thermogram showed an endothermic peak at 147.68°C in figure 6.2 which is in confirmation with the reported value M.P. Col 145 °C. [14]

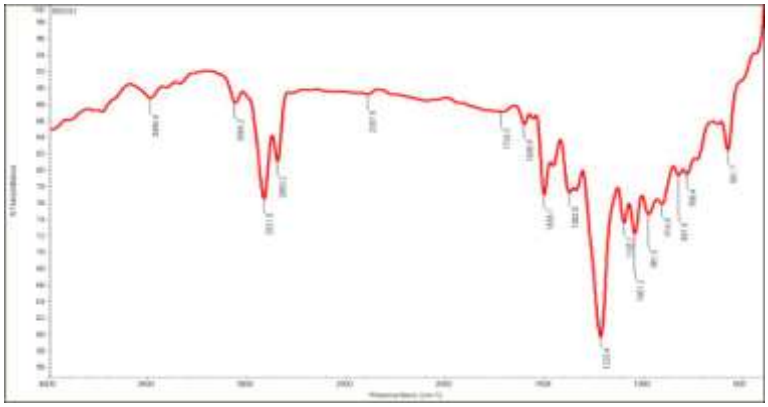

Fig. 4: DSC thermogram of drug Berberine

Solubility and partition coefficient:

Solubility in different solvents

Solubility profile of drug in different solvents at room temperature were shown in table 2

Table 2: Solubility of Berberine in different solvents

Solvent	Solubility	Inference
CHCl3	++	Highly Soluble

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CHCl3	+++	Highly Soluble
Ethanol	++	Slightly miscible
Acetone	++	Slightly miscible
Ether	++	Slightly miscible
Water	-	Insoluble

Drug excipients interaction

Interaction of drug, if any, with excipients was calculated by employing DSC analysis of the drug and drug excipients physical mixtures. Table 6.5 provides a summary of said pre-formulation outcomes that were so acquired.

Table 3: Pre-formulation observations of Berberine

Parameter	**Observation**
Physical appearance	Yellow crystalline powder
Assay for purity	99.53± 0.037%
Melting point	145ºC± 2 ºC
UV maxima in chloroform, acetate buffer	256 nm, 234 nm
Log P (n-octanol/water)	2.61±0.12

Optimization of parameters

Stirring speed

Average grain size and the impact of stirring speed, polydispersity and zeta potential were also optimized. Stirring time was fixed in the range of 500, 7500, 1000, and 1250 rpm and effect on size, PI % zeta potential were observed.

Table 4: Effect of stirring speed

Stirring speed (rpm)	**Mean particle size (nm) ±SD**	**Polydispersity index±SD**	**Zeta potential (-mV)**
500	115.4±6.01	0.312±0.016	-25.2±1.13
750	113.7±5.19	0.270±0.011	-26.1±1.21
1000	110±4.98	0.251±0.019	-32.9±1.14
1250	114±7.78	0.263±0.018	-26.4±1.21

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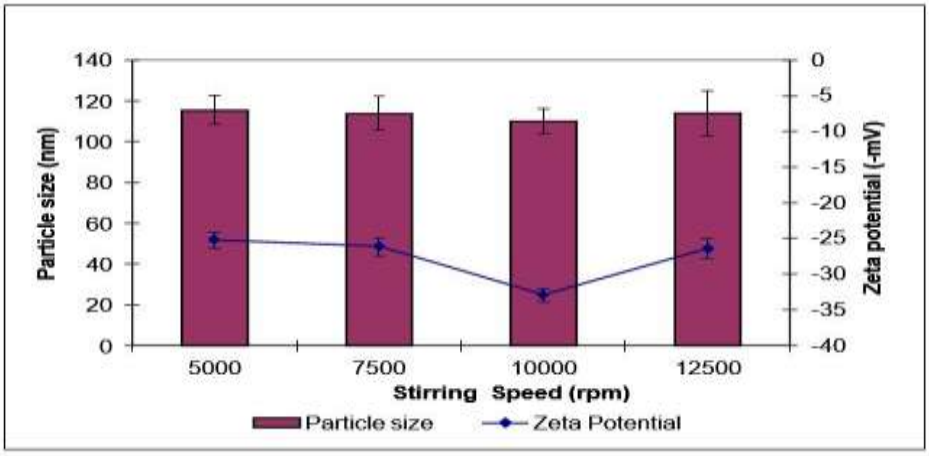

Fig. 5: Effect of stirring speed

Stirring time

Time is spent stirring has an impact on particle size, polydispersity and zeta potential were also optimized. Stirring time was fixed in the range of 01, 2.5, 05, and 7.5 min and effect on particle size, PI and zeta potential were observed. Results were presented here under.

Table 5: Effect of stirring time

Stirring time (min)	Particle size (nm)±SD	Polydispersity Index±SD	Zeta potential (-mV)
01	113.8±7.15	0.283±0.015	-27.5±1.13
2.5	113.4±8.27	0.261±0.013	-28.4±1.26
05	110±6.21	0.251±0.019	-32.9±0.95
7.5	116±10.84	0.263±0.015	-27.3±1.32

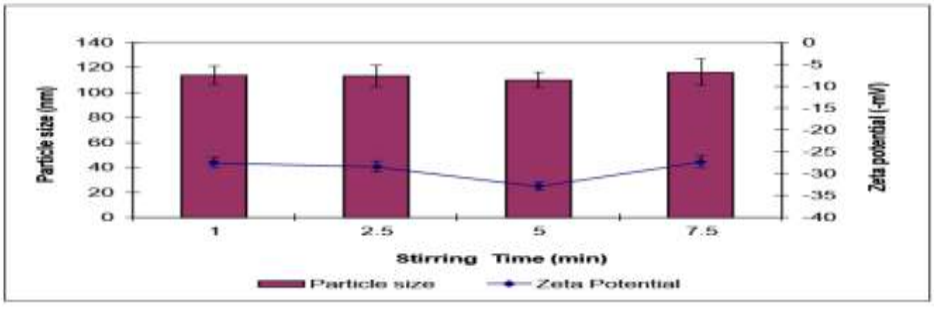

Fig 6: Effect of stirring time

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Sonication time

The relationship between particle size, polydispersity, & immobilization effectiveness was optimized as a function of ozonation time. Sonication time was fixed in the range of 02, 4, 06, 8 and 10min. Results were presented here under (Table 6 and Figure 7).

Table 6: Effect of sonication time

Sonication time (min)	Particle size (nm)±SD	Polydispersity Index±SD	%Entrapment efficiency	Zeta potential (-mV)
2	356.8±13.4	0.481±0.018	84.7±3.14	-28.2±1.13
4	248.3±7.15	0.357±0.015	87.1±2.19	-30.1±1.21
6	225.8±9.8	0.298±0.013	88.4±1.78	-29.5±2.18
8	**110±8.98**	**0.251±0.015**	**91.8±1.16**	**-32.9±1.14**
10	107±11.21	0.244±0.010	90.8±2.18	-31.5±2.27

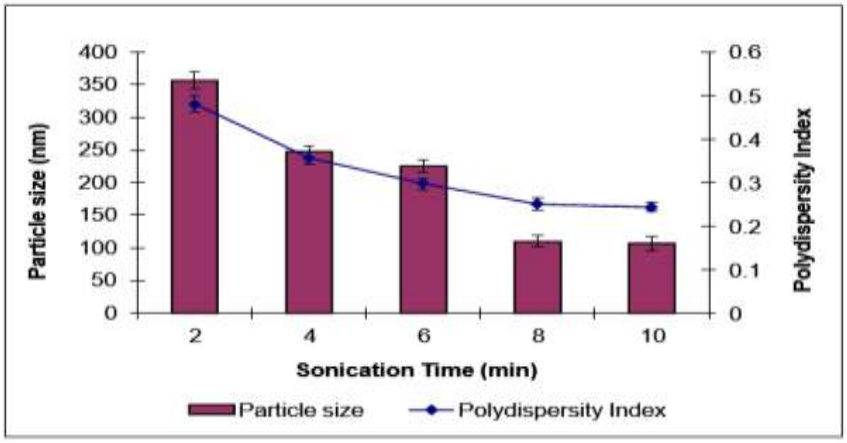

Fig 7: Effect of sonication time

Effect concentration of drug

Effect of varying dosage form on zeta potential, polydispersity, entanglement, and particle shape, among other characteristics had been measured. Drug concentration was fixed in the range of 01, 2.5, 5, and 7.5%. Results were presented here under.

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Table 7: Effect of drug concentration

% Drug concentration	Particle size (nm)±SD	Polydispersity Index ± SD	%Entrapment efficiency	Zeta potential (-mV)
1.0	102.3±11.96	0.298±0.016	93.27±2.13	-36.3±1.87
2.5	105±9.30	0.255±0.018	92.73±3.07	-31.1±1.95
5.0	110±9.98	0.251±0.015	91.8±2.19	-32.9±2.14
7.5	127.6±10.25	0.273±0.021	79.24±2.73	-29.8±2.81

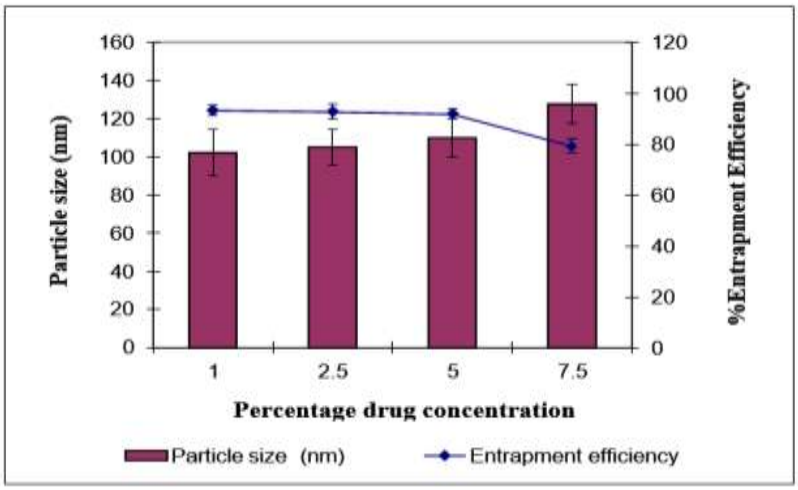

Fig 8: Effect of drug concentration

Particle shape and surface morphology

Electron microscopies (Both SEM and TEM) were carried out to study the surface morphology of the prepared nanoparticulate systems. SEM and TEM images of lipid particles reveal that SLN are ovoid to nearby spherical in shape but NLC are nearly spherical in shape. Photograph 6.12 shows SEM while 6.13 shows TEM images of drug loaded SLN dispersions respectively.

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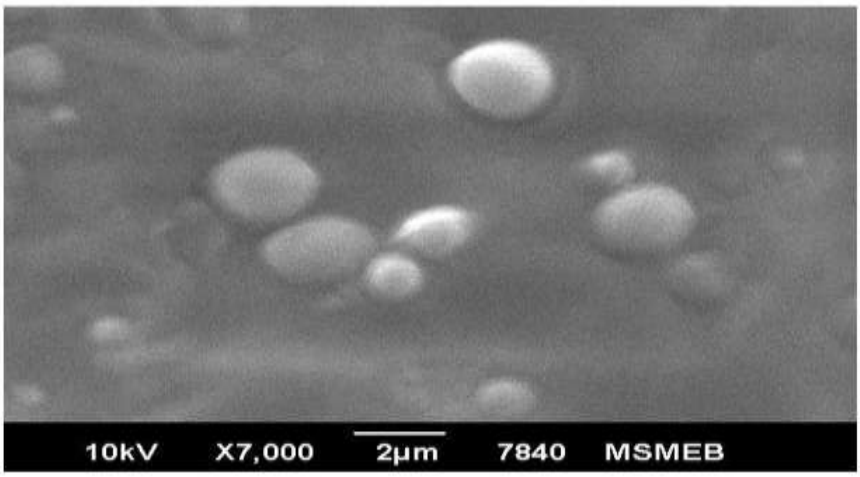

Fig9: SEM image of lyophilized NLS formulation

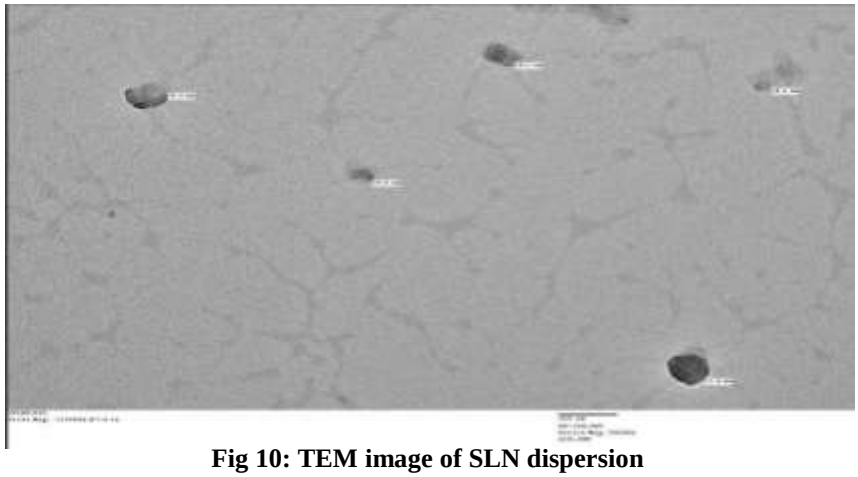

Fig 10: TEM image of SLN dispersion

Entrapment efficiency

Drug un-entrapped in the lipid nanoparticles were first separated from the systems and analyzed to determine entrapment of drug in NLC and SLN particles. Entrapment efficiency of dithranol was found to be 77.17±1.14% and 91.8±1.16% in SLN and NLC dispersions respectively while DL were 3.90% and 4.64% for SLN and NLC respectively.

In-vitro drug release

Data on the in vitro release of drugs of various formulations are shown in table 8 and graph 4.14 below.

Table 8In-vitro drug release of BRB loaded various formulations

Time (h)	Cumulative % drug release µg/cm^2 ± SD		
	Marketed Cream	SLN loaded gel	NLC loaded gel
1	0.91±0.81	6.50±2.20	9.0±1.91
2	2.78±2.08	11.13±2.16	13.50±1.67
4	4.72±2.6	18.5±2.25	21.02±2.06

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6	8.02±1.25	22.96±3.97	26.0±2.12
8	12.21±2.92	26.76±1.86	30.03±2.84
10	16.48±2.98	29.84±2.85	33.01±2.96
12	21.86±3.83	32.51±2.93	35.50±3.21
24	31.79±2.76	39.12±3.08	47.51±2.86

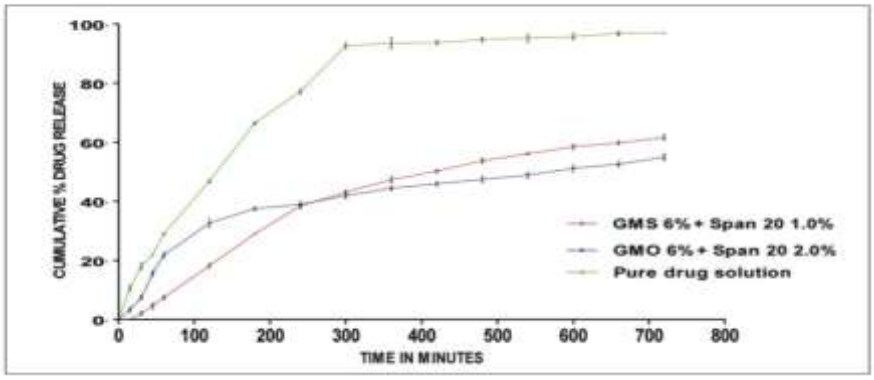

Fig 11: In vitro release of BRB loaded different formulations

Stability studies:

The findings demonstrated that the SLN dispersion held at 4°C had a higher entrapment efficiency than the SLN dispersion stored at 25°C 2°C. This might be because at higher temperatures, more drugs are expelled from the lipid matrix [Table 9].

Table 9: Investigations on the stability of SLNs (% of entrapment efficiency over 25°C and refrigerated temperatures)

Formulation	Immediately after	15th day (%)		30th day (%)	
	Preparation (%)	4 °C	25±2°C	4°C	25±2°C
F3	81.22	81.06	78.16	80.00	75.31
F6	85.70	85.06	82.90	83.94	77.91
F9	76.64	76.32	73.56	74.47	69.19
F12	77.59	77.02	74.02	75.81	70.01
F15	86.40	86.00	85.26	83.16	81.38
F18	82.71	82.15	81.86	81.57	77.69

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IV. CONCLUSION:

In order to improve BRB's aqueous solubility, stability, and oral bioavailability, the current work aimed to create and evaluate a stable nanoparticulate dosage of BRB (BRB-SLNs) along with in vitro and pharmacokinetic experiments. Based on DSC, UV, FTIR, NMR, and mass spectrum analyses, the drug was recognized. To assess how the physicochemical characteristics of the SLN were changed by systematically modifying formulation and process parameters, optimization of both process and formulation factors was carried out. 10% sucrose was used as a cryoprotectant to stabilize SLN dispersions during lyophilization, and the lyophilizates had good re-dispersibility. BRB has a pH-dependent dispersion, with increased solubility at extreme pH levels, according to pre-formulation experiments. Its highly hydrophilic characteristic was confirmed by BRB's poor solubility in organic solvents and extremely low evident partition coefficient at any and all pH levels. Ganciclovir was discovered to be stable in the liquid state, reaching its maximal stability at higher pH levels. BRB was stable both alone and in the company of excipients employed throughout the study period, according to solid state stability assessment drug-excipient compatibility analyses. Their eligibility for use in formulations was demonstrated by the lack of contact with the excipients. TEM, SEM, particle shape, zeta potential, morphologies and form, and crystallinity by differential scanning calorimetry were all employed to characterize the SLNs (DSC). Broadly speaking, these findings show that SLNs are showing promise delivery systems that can be created to increase the oral bioavailability of BRB. This will allow the dose of the pharmacological therapy to be decreased, the inconvenience of I.V. administration to be avoided, and patient compliance to be generally increased.

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