

Original Article

FORMULATION AND CHARACTERIZATION OF SOLID LIPID NANOPARTICLES CONTAINING GINGER OIL FOR ENHANCEMENT OF STABILITY

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ABSTRACT

Objective: To develop and characterize ginger oil loaded solid lipid nanoparticles (SLN) for enhancement of its stability.

Methods: Ginger oil loaded SLNs were prepared in four different batches by double emulsification method using different concentrations of soya lecithin and Tween 80. Further, these batches were characterized for particle size, zeta potential, drug entrapment efficiency and *in vitro* release study. After observing the results, batch F4 was further characterized by Fourier Transform Infrared Spectroscopy (FTIR), Transmission Electron Spectroscopy (TEM) and Differential Scanning Calorimetry (DSC). In addition the optimized batch was subjected to anti-microbial study. Finally, stability studies were done by storing the F4 formulation at accelerated condition, room temperature, refrigerated temperature and photostability were performed by exposing the formulation to UV/fluorescence lamp for 6 mo.

Results: The encapsulation efficiency of various batches of SLNs was in the range of 79.75 to 90.24%. The size ranges varied between 50 to 1000 nm. Zeta potential of all formulations was found to be in the range of -44.52 to -49.37 mV. The FTIR spectra of optimized F4 batch indicated no significant structural changes or complexation reactions between drug and excipients. Moreover, TEM image of displayed spherical shape with smooth surface. *In vitro* drug release study exhibited 95% drug release up to 12 h which indicated suitability of formulation. Thus F4 batch formulation stored at room temperature and refrigerated conditions was found most stable while, accelerated and photostability samples were found to be most susceptible in comparison.

Conclusion: The physicochemical stability of ginger oil extract was enhanced by loading it into solid lipid nanocarriers; the resulting SLNs also showed good antimicrobial potential against *Klebsiella pneumoniae* throughout storage conditions.

Keywords: Solid lipid nanoparticles, Ginger oil, Stability study, Antimicrobial study

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INTRODUCTION

Natural products have been used abundantly from ancient times due to their rich Phyto-constituents and lesser side effects [1]. The products from natural origin having complex mixtures are derived as raw or processed part of plant [2]. Most of the medicinal plants and phyto-preparations have been used in therapy and prevention of various human diseases, including the cardiovascular, gastrointestinal, nervous system, skin disorder and cancer [3].

Amongst the millions of medicinal products, Ginger (*Zingiber officinale* Roscoe, Zingiberaceae) is the one of the important prime herb in Ayurvedic, Chinese and Unani herbal medicines system. Since ancient times, it has been potentially used for the treatment of rheumatism, nervous disorders, gingivitis, toothache, asthma, stroke, constipation and diabetes [4]. Moreover, ginger had shown its effect in the prevention of post-operative nausea and vomiting without having significant gastric emptying and against cytokines secreted at the site of inflammation [5, 6]. In addition to this, ginger had proven its potential in emesis, hyperemesis gravidarum [7], motion sickness [8] and cancer chemotherapy [9].

Ginger is found to be a rich source of volatile oils, resinous matter, starch and mucilage [1]. It consists of about three percent of a fragrant essential oil, sesquiterpenoids in smaller amounts and a small monoterpenoid fraction [10]. The pungent principles of ginger produced hot sensation in the mouth and it was due to the occurrence of oleoresin in the form of gingerols, shogaols, paradols and zingerones [11].

Due to the increasing demand of ginger and its products in local and international markets, major emphasis was given on the development of stable formulation of ginger oil. However, the

formulations like ginger oil loaded emulsion were found susceptible to degradation, thus having less shelf life. To increase the shelf-life therefore, there is the needs of proper formulation of ginger oil like SLN, which might enhance its stability.

Solid lipid nanoparticles (SLNs) are lipid-based submicron-sized colloidal carriers systems and considered to amalgamate advantages of polymeric nanoparticles, liposomes and emulsions simultaneously decreasing their individual drawbacks [12-14]. Solid lipid core matrix present in SLNs can solubilize lipophilic molecules. The lipid core is stabilized by the use of surfactants (emulsifiers). There is the presence of physiological and compatible lipids with a high melting point as the solid core, which is coated by non-toxic amphiphilic surfactants as the outer shell. The advantage of SLN formulation lies in their submicron size range (50-1000 nm) and existence in the solid-state at both body and room temperatures [15]. Number of unique properties of SLNs makes them the convincing option for improving the performance of pharmaceuticals, nutraceuticals [16].

The stability testing of Solid lipid nanoparticles is equally important as it has the following potential applications in quality attributes like compatibility of the container closure system, comparability assessment after manufacturing changes, expiration dating, stability data and product specification.

Thus above study was aimed to develop ginger oil loaded solid lipid nanoparticles and demonstrate the stability of the formulation under accelerated conditions, room temperature, refrigerated and photostability conditions. It was expected that shelf life of ginger oil will be enhanced and further, the formulation was also studied for antimicrobial activity.

MATERIALS AND METHODS

Materials

Ginger Volatile oil was obtained as a gift sample from Nisarg Biotech, Satara. Soya lecithin was purchased from VAV life sciences, Mumbai. Cholesterol was obtained from Loba Chemie and Polyvinyl alcohol from SD Lab, Mumbai. Potassium dihydrogen phosphate, KBr IR grade, NaOH Pellets, Dichloromethane, Tween 80 were purchased from Merck specialties Pvt. Ltd. Methanol was obtained from Molyche Molychem Ltd. and Ethanol from Changshu Yangyan Chemicals, China.

Methods

Preparation of ginger oil loaded solid lipid nanoparticles

Double emulsification method was used to prepare ginger oil loaded SLNs. Soya lecithin and cholesterol as lipids while low molecular weight PVA as hydrophilic polymers was chosen after preliminary trials for the selection of polymers. Ginger oil was dissolved in methanol while soya lecithin and cholesterol were dissolved in dichloromethane. Both solutions were stirred for 30 min to

solubilize polymers and drug. Then organic solution was added dropwise to the aqueous portion by syringe. Tween 80 was added as stabilizer and homogenized for 10 min. Later the white cloudy primary emulsion was poured into 2% PVA solution and homogenized for an additional 5 min. The resultant w/o/w emulsion was stored at room temperature and the solvent was evaporated in rotavac. Further formulation was sonicated on high-intensity probe sonicator for 5 min to reduce the size. The stable emulsion was freeze-dried at -80 °C under reduced pressure and lyophilized to get SLNs [17, 18].

Characterization of SLN

Particle size determination

Particle size analysis was performed by using the laser diffraction technique (Malvern 2000 SM, Instruments, UK). The particle size measurements were carried out at a 90 ° scattering angle. The SLN sample was dispersed in distilled water and the average particle size was determined. The data presented was mean values of three independent samples produced under identical production conditions.

Table 1: Formulation of ginger oil loaded solid lipid nanoparticles

Ingredients	F1	F2	F3	F4
Ginger volatile oil	20 mg	20 mg	20 mg	20 mg
Soya lecithin	20 mg	40 mg	50 mg	100 mg
Cholesterol	10 mg	10 mg	10 mg	10 mg
Dichloromethane	10 ml	10 ml	10 ml	10 ml
Tween 80	1%	2%	3%	4%
PVA	2%	2%	2%	2%
Water	q. S	q. S	q. S	q. S

Zeta potential

Photon Correlation Spectroscopy (PCS) (Malvern Zetasizer UK and ZEN 3600) was used to determine zeta potential of SLNs [19]. The samples were diluted in the ratio of 1:20 with deionized water. All results correspond to the average±SD of three separate experiments at 25 °C.

Determination of drug entrapment efficiency

The entrapment efficiency was calculated by using 2 g of formulation. The solution was centrifuged at 12000 rpm for 20 min. Then supernatant fluid was collected and passed through the membrane filter. 1 ml of supernatant was then taken and diluted to 10 ml with ethanol. The supernatant was assayed spectrophotometrically at 230 nm [17].

Encapsulation efficiency or the entrapment efficiency was determined by using the formula given below:

$$\%EE = CA/WA * 100$$

CA is the total concentration of ginger oil loaded solid lipid nanoparticles;

WA is the total amount of ginger oil added;

Drug content

The drug content of SLN was determined by dissolving SLN solution in ethanol and the absorbance by UV visible spectrophotometer at 230 nm. The drug content was calculated as

$$\%DC = CA * (VA / WA) * 100$$

Where:

DC is drug content

CA is the total concentration of ginger oil loaded solid lipid nanoparticles;

WA is the theoretical amount of ginger oil added;

VA is the volume of solid lipid nanoparticles.

In vitro release study

In vitro release studies were performed using the dialysis bag method, modified to maintain a sink condition and achieve satisfactory reproducibility. The dialysis bag was soaked in deionized water for 12 h before use. Ginger oil loaded SLN dispersion was first poured into the dialysis bag with the two ends fixed by a thread and placed into the preheated dissolution media (phosphate buffer pH 7.4) placed in a beaker. The beaker was placed on a magnetic stirrer. At fixed time intervals of 1 hour samples were withdrawn for analysis and an equal volume of fresh dissolution medium was added until up to 12 h. The samples were filtered in membrane filter (0.22 µm) it was analyzed by using a UV Spectrophotometer at a λmax of 230 nm against phosphate buffer of pH 7.4 as blank [20].

Anti-microbial studies using well diffusion method

Well diffusion method described by Perez *et al.* was used for assessment of antibacterial activity of ginger oil loaded SLNs [21]. Samples of SLNs and standards were prepared using mixture of ethanol in deionized water. Nutrient broth was used for the preparation of culture of pathogenic bacteria (Hi media, India). 1% of pathogenic bacteria (10⁹ CFU/ml) were added and mixed in Nutrient Agar at 40 °C. NA with 1% of pathogenic bacteria was poured to Petri plates. Wells were prepared by sterile cork borer and left to dry at room temperature. After that, prepared SLNs and standards formulations (5 mg/ml) were poured to the wells individually and kept at 37±2 °C for 24 h. Inhibition zone diameter was carefully measured, and values were expressed as mean with standard deviation. The experiment was done in triplicate. Further antimicrobial potential of F4 formulation stored at different stability conditions was estimated at the end of six months using a similar procedure as stated above.

Optimization of formulation

After observing the desired results of particle size, zeta potential, drug content and entrapment efficiency, the final batch was optimized.

IR spectrum of SLN

The samples were mixed with 100 mg of potassium bromide (KBr). The samples were compressed to disc by applying the pressure of 5 tons for 3 min in a hydraulic press. The prepared pellets were placed in the sample cell and the spectrums were analyzed in the region of 4000-400 cm^{-1} . By comparing the spectrums of ginger oil and formulation, the compatibility study was performed.

Surface morphology

Surface morphology of solid lipid nanoparticles loaded ginger oil was done by Transmission Electron Microscope (TEM) [22]. A small amount of SLN was taken in the metal stub. The stub was coated with conductive gold by Hitachi 1010 ion sputter and observed under Hitachi 3000 N Transmission electron microscope (JSM 5610 LV SEM, JEOL, Japan) chamber. The image was scanned at an acceleration voltage of 20 kV with a chamber pressure of 0.8 mmHg.

Differential scanning calorimetry

The thermal characteristics were determined using a differential scanning calorimeter (DSC Mettler Toledo).

Approximately 10 mg of the samples were placed in aluminum pans with lids and heated. An empty aluminum pan was used as the reference. Differential scanning calorimetric curves were recorded in pure ultrahigh dry nitrogen across a temperature range of 30 $^{\circ}\text{C}$ –180 $^{\circ}\text{C}$, with a constant linear heating rate of 10 $^{\circ}\text{C}$ per minute. Finally, the enthalpies were calculated using the Mettler Star software.

Stability studies

Stability studies were carried out as per ICH norms for six months using Programmable environmental test chambers (Thermolabs Instruments Ltd.). The optimized formulation (F4) was packed in amber-colored vials and closed with airtight closures and stored for six months under different conditions of temperature and/light and labeled according to storage conditions. Samples were analyzed every month and were evaluated for appearance, pH, polydispersity index and entrapment efficacy up to the fifth month. At the end of the sixth month the samples were analyzed for particle size, antimicrobial activity, *in vitro* dissolution study and entrapment efficiency.

Table 2: Form codes of an optimized batch for different storage condition

S. No.	Test conditions	Temperature conditions	Form code
1	Accelerated	40 $^{\circ}\text{C}\pm 2$ $^{\circ}\text{C}/75\%\pm 5\%$ RH	A
2	Room temperature	25 $^{\circ}\text{C}\pm 2$	B
3	Refrigerated	<4 $^{\circ}\text{C}\pm 1$	C
4	Photo Stability	UV/fluorescence lamp	D

Phase separation study

Phase separation study was carried out at a storage period of 1, 3, and 6 mo to check compatibility and stability of formulations by using distilled water and phosphate buffer saline. 0.5 ml of each test sample was added to 5 ml of distilled water in the centrifuge tube and it was centrifuged at 4000 rpm for about 10 min. The study was repeated using saline phosphate buffer instead of phosphate buffer. It was visually observed after 1 hr of centrifugation for any phase separation or turbidity.

RESULTS

Characterization of SLNs

Particle size

Particle sizes of SLNs were observed in the range of 200 to 1000 nm, which was mostly affected by the amount of Tween 80 (table 3). The particle size distribution of batch F4 for d(10%), d(50%) and d(90%) were found to be 71 nm, 109 nm and 182 nm, respectively, while the polydispersity index was found to be 0.644 respectively [23]. Particle size was impacted by increased concentration of surfactant

as at higher concentration, the amount of surfactant was sufficient to cover primary emulsion [24, 25].

Zeta potential determination

Zeta potential of four batches was observed in the range of -44.52 to -49.37 indicating stability and charge distribution (table 3). Negative charge was probably due to the outer shell formed by nonionic PVA.

Entrapment efficiency

The entrapment efficiency of the ginger oil loaded SLNs was in the range of 79 to 90% (table 3), which were mainly influenced by the concentration of lipid and surfactant. Entrapment of drug was observed to be increasing with the increasing concentration of Tween 80 and soya lecithin, which might be responsible for the prevention of leaching of ginger oil from SLNs [24].

Drug content

The drug content of the ginger oil loaded SLN was in the range of 82 to 94% (table 3). Drug content was mainly influenced by the concentration of tween 80, which is stabilizer and soya lecithin with the drug for the formation of core and core modification in preparation of SLN.

Table 3: Particle sizes, polydispersity index, zeta potential, drug content and entrapment efficiency of F1 to F4 formulation

Batch	Particle size (nm)	Polydispersity index (PDI)	Zeta potential	Drug content (%)	Entrapment efficiency (%)
F1	209 \pm 2	0.984 \pm 0.06	-44.52 \pm 3.1	82.21 \pm 2.3	79.75 \pm 0.85
F2	197 \pm 3	0.913 \pm 0.09	-47.28 \pm 3.2	87.08 \pm 3.5	86.02 \pm 0.75
F3	187 \pm 2	0.785 \pm 0.21	-47.93 \pm 4.2	90.18 \pm 3.6	89.05 \pm 0.81
F4	182 \pm 2	0.644 \pm 0.22	-49.37 \pm 3.9	94.41 \pm 2.8	90.24 \pm 0.54

(n=3, mean \pm SD)

In vitro drug release study

The drug release of all the formulations was studied. The effect of the polymer ratio on the release of the drug from the SLN was studied and compared within four batches. The F4 batch gives better drug release than other batches. The drug release of all formulations (F1-F4) is shown in fig. 1. The solid lipid nanoparticles show an initial burst release (1 h) of 32.76-41.53.05%. The F4 formulation depicted high drug release due to the high concentration of lipid and

surfactant ratio. At the end of 12 h, the percentage of drug release was observed around 95.22%: the F4 formulations depicted higher and prolonged drug release over other formulations.

Optimization of formulation

After observing the desired results of particle size, zeta potential, drug content and entrapment efficiency F4 batch were selected to carry out further stability studies.

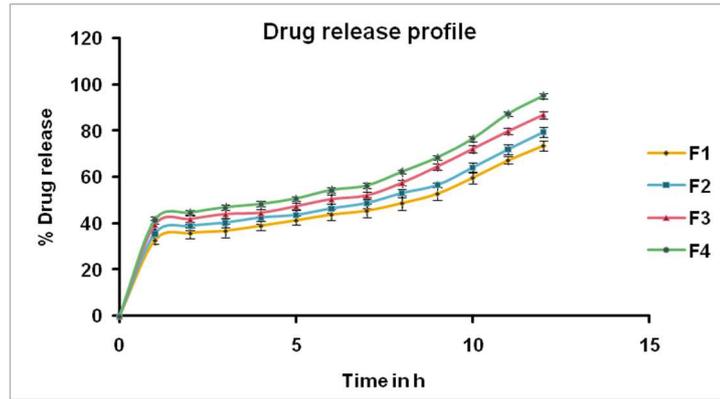


Fig. 1: Cumulative release of ginger oil loaded SLNs of F1, F2, F3 and F4 formulations, (n=3, mean±SD)

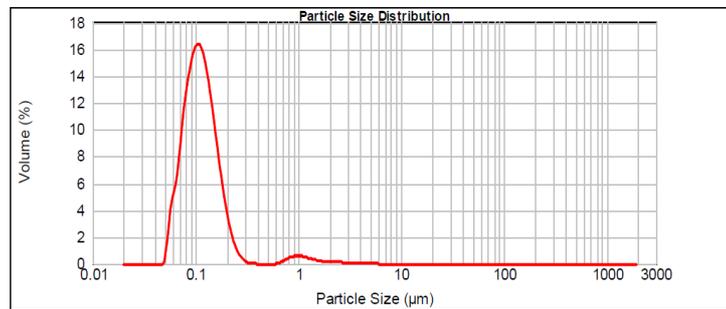


Fig. 2: Particle size distribution of an optimized batch

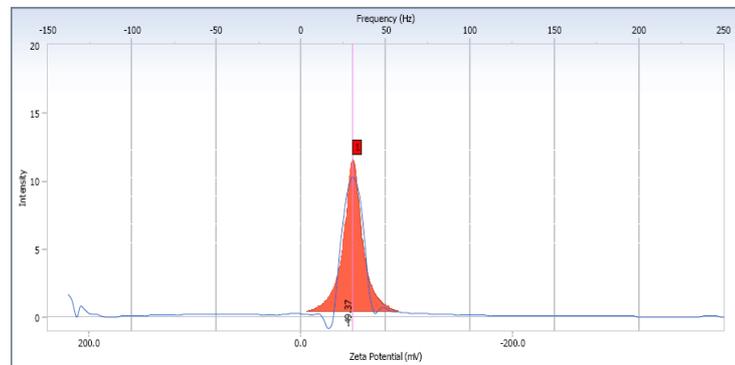


Fig. 3: Zeta potential determination of optimized batch

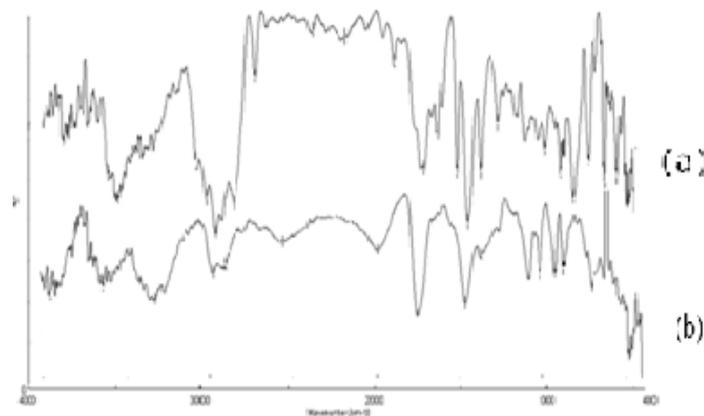


Fig. 4: FTIR spectra of (a) ginger oil (b) formulation

Fourier transforms infrared spectroscopy

The characteristic peaks indicating the functional groups such as 2931 cm^{-1} for OH stretch; H-bonded and 1370 cm^{-1} CH₃ bond which was essential for activity, remained unaltered and lower in the intensity in the formulation spectra. Pure drug indicated a prominent peak at wave number $1270\text{--}1718\text{ cm}^{-1}$. Formulation F4 showed all characteristic peaks of drug indicating there was no chemical interaction between drug and components selected. However, peaks in the formulation were observed to have reduced intensity.

Transmission electron microscopy

The solid lipid nanoparticles loaded ginger oil optimized (F4) Formulation were observed to be a spherical shape with smooth surface (fig. 5). The credit of formation of spherical shaped SLNs might be given to partitioning of organic solvent into aqueous medium followed by lipid precipitation around drug and simultaneous evaporation of solvent entrapped.

Differential scanning calorimetry

In (fig. 6) 16 graph (b) showed a broad endothermic peak at $103\text{ }^{\circ}\text{C}$. Another graph (a) is the formulation graph; this graph also showed an endothermic peak at $101\text{ }^{\circ}\text{C}$. Therefore, that formulation was claimed to be compatible with the polymer. From these results it was clear that the formulation was stable, as there was no significant change in its thermal properties [26]

Antimicrobial study

The degree of the zone of inhibition was mainly dependent on the type of bacteria as well as the type of formulation. F4 formulation depicted a bigger diameter of growth inhibitory zones compared to others [fig. 7]. Among the bacterial strains, *Klebsiella pneumoniae* was easily inhibited by all the tested samples followed by *Staphylococcus aureus* while *Escherichia coli* were found most difficult to inhibit [27].

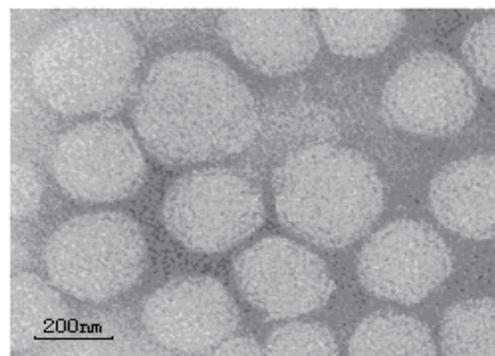


Fig. 5: TEM image of optimized formulation

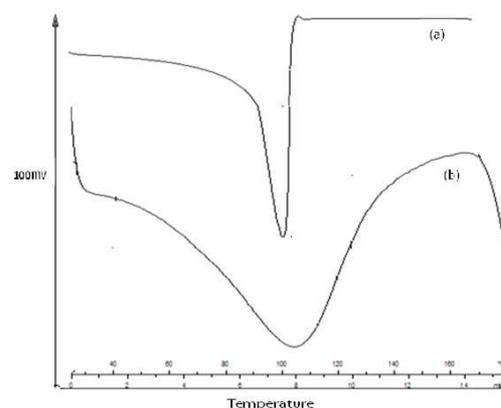


Fig. 6: Thermo-grams of formulation and soya lecithin

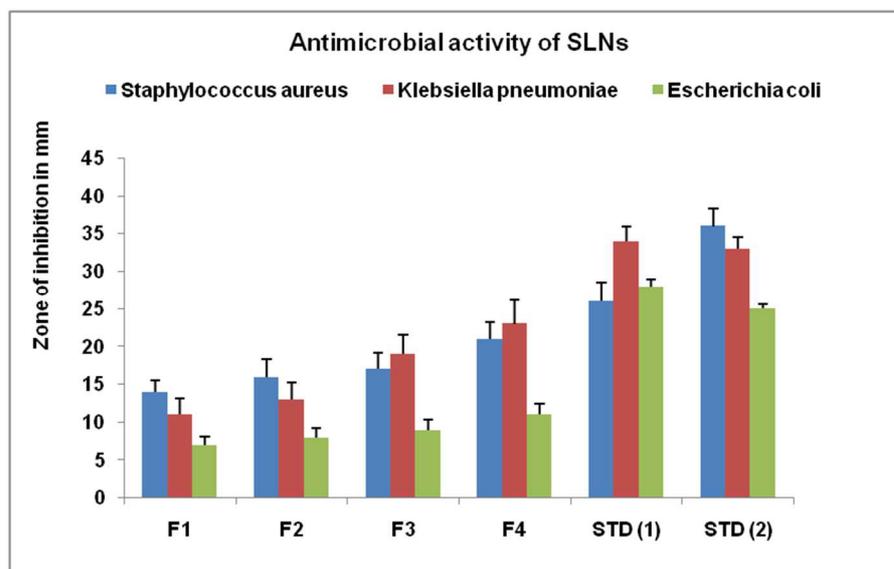


Fig. 7: Diameters of the zone of inhibition of F1 to F4 batches against *Staphylococcus aureus*, *Klebsiella pneumoniae*, *E. Coli* (n=3, mean±SD), STD (1): Ciprofloxacin STD (2): Ampicillin

Stability studies

Physical changes of formulation during stability

The stability studies on optimized formulation code F4 were carried out for a period of six months (table 4). The F4 batch was studied for physical characteristics at different stability storage conditions at one-month intervals. In accelerated and photostability conditions, it was seen

that up to month four there was no change in the physical characters. However, at the end of month five and six, formulations started to show some visible particles. However, the slight phase separation was observed in accelerated condition and the complete phase was separated in photostability condition. The appearance was still whitish translucent. In-room temperature and refrigerated condition, there was no any change in the physical characters at the end of six months.

Table 4: Physical changes in optimized batch in different stability conditions

Form code	Initial	1 st month	2 nd month	3 rd month	4 th month	5 th month	6 th month
A		+	+	+	+	-	-
B	White and	+	+	+	+	+	+
C	clear liquid	+	+	+	+	+	+
D		+	+	+	+	-	-

Note: +means there was no change

Determination of the effect of pH on stability

pH of formulation F4 was measured at an interval of one month for about six months and comparison was made between F4 batch stored at different stability conditions (table 5). This study revealed

that the F4 batch stored at room temp was stable up to six months while those stored at refrigerated temp was stable up to five months. The F4 batch stored at accelerated and photostability temp was stable up to three months, wherein, the pH had changed from 7.2 to 7.9.

Table 5: Changes in pH during different stability conditions

Formulation codes	Initial	1 st Month	3 rd Month	5 th Month
A		7.2±0.2	7.5±0.1	7.9±0.2
B	7.2±0.1	7.2±0.3	7.2±0.2	7.2±0.2
C		7.2±0.2	7.2±0.2	7.3±0.3
D		7.2±0.2	7.4±0.3	7.8±0.2

(n=3, mean±SD)

Determination of effect of particle size distribution on stability

Polydispersity index for F4 batch was studied for 6 mo. It was observed that the polydispersity index was gradually increased from

0.644 to 0.824 and 0.724 for accelerated and room temperature, while it was altered to 0.741 and 0.913 for refrigerated and photostability conditions. Entrapment efficiency was observed to be least in D batch at the end of the 5th month.

Table 6: Particle sizes of optimized formulation stored at different stability conditions

Formulation codes	Initial	1 st Month	3 rd Month	5 th Month
A		0.640±0.14	0.720±0.10	0.824±0.14
B	0.644±0.22	0.645±0.12	0.665±0.14	0.724±0.21
C		0.653±0.20	0.682±0.22	0.741±0.12
D		0.698±0.21	0.834±0.16	0.913±0.15

(n=3, mean±SD)

Table 7: Entrapment efficiency of optimized formulation stored at different stability conditions

Formulation codes	Initial	1 st Month	3 rd Month	5 th Month
A		89.25±0.32	86.92±0.21	85.47±0.25
B	90.24±0.54	90.21±0.51	87.21±0.36	83.66±0.36
C		89.21±0.36	84.32±0.24	82.36±0.42
D		89.33±0.32	83.64±0.25	81.69±0.26

(n=3, mean±SD)

Phase separation study

Phase separation study was carried out at a storage period of one, three and six months to check compatibility and stability of F4 batch formulation in distilled water and phosphate buffer saline (table 8 and 9). It was found that F4 batch stored at accelerated and

photostability exhibited phase separation end of the month; however, F4 batch stored at room temp and refrigerated did not show phase separation up to six month. In this study, F4 formulation batch stored at room temperature and refrigerated was found to be substantially stable as there was no phase separation and found to be clear.

Table 8: Phase separation study in distilled water of optimized formulation stored at different stability conditions

Formulation codes	At the end of 1st month	At the end of 3rd month	At the end of 6th month
A	No phase separation	No phase separation	Phase separation
B	No phase separation	No phase separation	No phase separation
C	No phase separation	No phase separation	No phase separation
D	No phase separation	Slightly phase separation	Phase separation

Table 9: Phase separation study in phosphate buffer saline of optimized formulation stored at different stability conditions

Formulation codes	At the end of 1st month	At the end of 3rd month	At the end of 6th month
A	No phase separation	No phase separation	Phase separation and showed particles
B	No phase separation	No phase separation	No phase separation
C	No phase separation	No phase separation	No phase separation
D	No phase separation	Slight phase separation and shown some particles	Phase separation and shown some particles

Particle size, polydispersity index, entrapment efficiency, *in vitro* dissolution and antimicrobial activity at the end of six months

Particle size of optimized batch stored at different stability conditions was observed in range of 194 to 235 nm, being higher for the accelerated condition. However, the polydispersity index value

was higher in case of photo-stability condition ranging from 0.756 to 0.989. Entrapment efficiency was reduced considerably in accelerated conditions, while antimicrobial activity was retained to a considerable extent at all storage conditions. Drug release studies revealed better drug release in formulation stored at room temperature and refrigerated stability conditions as compared to those at accelerated and photo-stability conditions [26].

Table 10: Particle sizes, polydispersity index and entrapment efficiencies of optimized formulation stored at different stability conditions at the end of 6 mo

Formulation codes	Particle size (nm)	Polydispersity index	Entrapment efficiency (%)
A	235±3	0.951±0.05	76.86±0.87
B	194±2	0.756±0.23	81.54±0.78
C	220±1	0.799±0.12	80.87±0.58
D	215±5	0.989±0.06	79.52±0.69

(n=3, mean±SD)

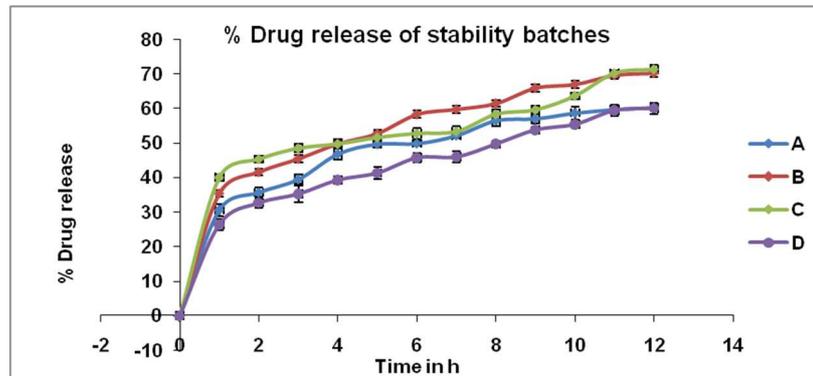


Fig. 8: Cumulative release of optimized formulation stored at different stability conditions, (n=3, mean±SD)

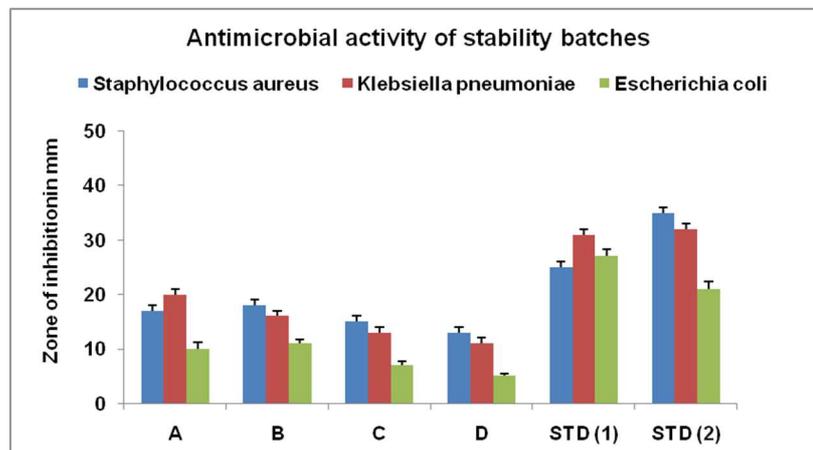


Fig. 9: Diameters of zone of inhibition of F1 to F4 batches against *Staphylococcus aureus*, *Klebsiella pneumoniae*, *E. Coli*, (n=3, mean±SD), STD (1): Ciprofloxacin STD (2): Ampicillin

DISCUSSION

The solid lipid nanoparticles of ginger oil were formulated by a double emulsification technique. The interfacial tension between the phases was minimized with the help of Tween 80. The stability of formed w/o type emulsion was enabled by adding co-emulsifier PVA as a hardening agent. The entrapment efficiency was influenced by the increased concentration of Lecithin and Tween 80. The DSC and FTIR spectrums revealed an absence of drug and lipid incompatibility. Higher entrapment of ginger oil in lipid core was represented by F4 formulation owing to its higher lipid content. Tween 80 was key ingredient responsible for reducing interfacial

tension during homogenization and approximate spherical shaped solid lipid nanoparticles. Moreover, the concentration of tween 80 was found to be inversely related to particle size as at higher concentration, SLNs exhibited lesser size. At lower concentrations, due to the lower amount of surfactant, it was unable to cover the dispersion, thus giving system less stability [27]. Partitioning of organic solvent in an aqueous medium resulted in lipid precipitation around ginger oil. However, the entrapped organic solvent was evaporated, simultaneously assuming the formed SLNs of spherical shape. F4 formulation revealed the release of the drug over a prolonged period of time, with the complete release after 12 h. Interestingly initial burst release in the formulation was due to

adsorption of some amount of drug on the surface of SLNs, while hindering resistance of lipid shell around the drug could be claimed as one of the reasons for later sustained release [27]. Best fit model for drug release of ginger oil loaded SLNs was found to be Korsmeyer Peppas model with R^2 value from 0.9939 to 0.9941, revealing combined diffusion and hydrogel relaxation as the mechanism of drug release [29]. Amongst all bacterial strains, highest antimicrobial activity was shown against *Klebsiella pneumoniae* and least against *E. Coli* [28].

Instability studies, slight phase separation was observed in accelerated condition due to increased kinetic energy of system, while in photo-stability condition, complete phase separation depicted susceptibility of SLNs to light. Polydispersity index, which is measure of heterogeneity of particle sizes, was observed to be increased again in accelerated and photo-stability condition, increased aggregation of particles due to collision due to more kinetic energy being the predicted reason behind.

At the end of sixth month, particle size and polydispersity index was more in almost all formulations stored at different storage conditions being highest in photo-stability and accelerated conditions. Lower entrapment efficiency at the end of six months revealed leakage of drug molecules from lipid core. Antimicrobial activity was observed to be retained in formulations stored at all storage conditions, but was on similar line for accelerated and photo-stability conditions. Thus, enhanced solubility of ginger oil in lipid matrix and decreased electrostatic repulsion between dispersed particles contributed to the long-term stability of SLNs, compared to ginger oil alone [27, 30].

CONCLUSION

In this study, the potential of SLNs dispersions as carriers for the delivery of ginger oil was exploited. Solid lipid Nanoparticles were prepared by the w/o/w type double emulsification method by using bio-acceptable lipids such as Cholesterol, soya lecithin and Tween 80 as an emulsifier. Drug loaded SLNs revealed average diameters in the narrow colloidal size range, a good loading capacity and drug release. The present formulation of solid lipid nanoparticles loaded ginger oil extract was found to be in the nano-size range. The physicochemical stability of ginger oil was enhanced by loading into solid lipid nanocarriers within established specification maintaining its integrity, quality and activity throughout the storage conditions. Moreover, ginger oil loaded SLNs could serve as an antimicrobial agent by retaining its potential by means of an intact colloidal carrier system.

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All authors have contributed equally in the research work.

CONFLICT OF INTERESTS

The authors declare no conflict of interest.

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