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FORMULATION AND IN VITRO EVALUATION OF METHOTREXATE -LOADED SOLID LIPID NANOPARTICLES

Kethavath Deepthi[1], Sayeeda Naseer[2] ,Shaik Sami[2], Netula Shiva Chandu[2],Kuruva Shiva Kumar[2],Nenavath ShivaLeela[2]

[1]Assistant Professor, Department of Pharmaceutics, Teegala Ram Reddy college of pharmacy, Meerpet, Hyderabad, Ranga Reddy Telangana - 500097, India.

[2]Students, Teegala Ram Reddy college of pharmacy, Meerpet ,Hyderabad, Ranga Reddy Telangana - 500097, India.

Abstract:

The present study was aimed at the formulation and in vitro evaluation of Methotrexate-loaded Solid Lipid Nanoparticles (SLNs) to enhance drug delivery, improve stability, and achieve controlled release. Methotrexate, a poorly water-soluble anticancer drug, exhibits limited bioavailability, which can be improved through nanocarrier-based systems. SLNs were prepared using glyceryl monostearate as the lipid matrix and Tween 40/Span 80 as surfactants by a suitable lipid-based formulation technique. Four formulations (F1–F4) were developed and evaluated for physicochemical properties, particle size, zeta potential, entrapment efficiency, in vitro drug release, and stability studies. FTIR studies indicated no significant drug–excipient interactions, confirming compatibility. The optimized formulation (F4) exhibited the smallest particle size (119 nm), highest entrapment efficiency (85.6%), and highest zeta potential (-25.8 mV), indicating improved stability and effective drug encapsulation. In vitro release studies demonstrated sustained drug release up to 8 hours, with F4 showing maximum release (97.53%). Stability studies confirmed that the optimized formulation remained stable under different storage conditions over three months with minimal variation in drug content. The study concluded that SLNs are a promising drug delivery system for Methotrexate, significantly improving its release profile, stability, and encapsulation efficiency. Formulation F4 was identified as the optimized batch based on overall performance.

Keywords: *Methotrexate, Solid Lipid Nanoparticles, Drug Delivery System, Sustained Drug Release, Entrapment Efficiency.*

Corresponding author:

Kethavath Deepthi,

Assistant professor, Department of Pharmaceutics, Teegala Ram Reddy College of Pharmacy, Hyderabad - 500097, Telangana, India.

Mobile:

Email Id:

QR CODE

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INTRODUCTION:

Solid lipid nanoparticles (SLNs) are advanced colloidal drug delivery systems that have gained significant attention in pharmaceutical research due to their ability to improve drug stability, bioavailability, and therapeutic efficacy. SLNs were first introduced in the early 1990s as an alternative to traditional carrier systems such as liposomes, emulsions, and polymeric nanoparticles. They are composed of physiologically compatible lipids that remain solid at both room and body temperatures, stabilized by suitable surfactants .SLNs typically range in size from 50 to 1000 nm and consist of a solid lipid core matrix in which the drug is either dissolved or dispersed. This solid matrix enables controlled and sustained drug release while protecting the incorporated drug from chemical degradation. Due to their unique properties, SLNs are particularly suitable for delivering poorly water-soluble drugs, such as Paclitaxel, enhancing their solubility and bioavailability. One of the major advantages of SLNs is their biocompatibility and biodegradability, as they are prepared using physiological lipids such as triglycerides, fatty acids, and waxes. Additionally, SLNs avoid the use of toxic organic solvents in many preparation methods, making them safer for clinical applications. Their ability to provide targeted drug delivery, reduce systemic toxicity, and improve therapeutic outcomes has made them a promising approach in modern drug delivery systems. Furthermore, SLNs have shown great potential in various routes of administration, including oral, topical, parenteral, and pulmonary delivery. Their versatility, ease of large-scale production, and stability during storage contribute to their growing importance in pharmaceutical development. As a result, SLNs are widely explored for applications in anticancer therapy, gene delivery, and controlled drug release systems .

Structural Components of Solid Lipid Nanoparticles (SLNs)

1. Solid Lipid Core

The solid lipid core is the fundamental structural component of SLNs, consisting of lipids that remain solid at both room and physiological temperatures. Commonly used lipids include triglycerides (e.g., tristearin), fatty acids (e.g., stearic acid), partial glycerides, and waxes. This core serves as the matrix for drug incorporation, where the drug can either be dissolved or dispersed within the lipid structure. The solid nature of the lipid matrix provides controlled and sustained drug release and protects the drug from chemical degradation .

The crystallinity and polymorphic nature of the lipid significantly influence drug loading and release behavior. Highly ordered lipid structures may lead to drug expulsion during storage, while less ordered matrices can accommodate higher drug amounts.

2. Drug

The drug is the active pharmaceutical ingredient incorporated into the SLN system. Depending on its physicochemical properties, the drug may be either molecularly dispersed or present as amorphous clusters within the lipid core. SLNs are particularly suitable for lipophilic drugs such as Paclitaxel, which exhibit poor aqueous solubility.

Drug distribution within SLNs can follow different models such as homogeneous matrix, drug-enriched shell, or drug-enriched core. This distribution affects the release profile, where uniform dispersion leads to sustained release, while surface localization may result in burst release.

3. Surfactant / Emulsifier Layer

Surfactants or emulsifiers are essential for stabilizing SLNs by reducing interfacial tension between the lipid and aqueous phases. They form a protective layer around the lipid core, preventing aggregation and ensuring physical stability of the nanoparticles. Common surfactants include Tween 80, Poloxamer 188, lecithin, and sodium cholate.The type and concentration of surfactant influence particle size, surface charge, and stability of SLNs. Adequate surfactant concentration ensures uniform particle distribution, while insufficient amounts may lead to aggregation or coalescence.

4. Aqueous Dispersion Medium

The aqueous dispersion medium is the external phase in which SLNs are dispersed. It typically consists of purified water along with stabilizers or preservatives. This medium facilitates nanoparticle formation during preparation and contributes to the overall stability of the formulation.The properties of the aqueous phase, such as pH, ionic strength, and viscosity, can affect particle size distribution and zeta potential. Proper optimization of the aqueous medium is necessary to maintain long-term stability and prevent particle aggregation.

MATERIALS:

Methotrexate was procured from Synpharma Research Labs, Glyceryl monostearate, Tween 80 and Span 20, were obtained from Synpharma Research Labs, Hyderabad. Other chemicals and the reagents used were of analytical grade.

METHODOLOGY:

Method of preparation of Methotrexate loaded solid lipid nanoparticles:

Preparation of solid lipid nanoparticle formulations of Methotrexate by hot homogenization method

The solid lipid (Glyceryl monostearate) was melted above its melting point. The drug (Methotrexate) was dissolved or dispersed in the molten lipid. An aqueous surfactant solution (Tween 40 and Span 80)

was heated to the same temperature. The lipid phase was added to the aqueous phase under high-speed homogenization to form a coarse emulsion. The emulsion was subjected to ultrasonication for a specified time to reduce particle size. The nano emulsion was cooled to room temperature to allow lipid solidification and formation of SLNs.

Table -1: Composition of Methotrexate for preparation of solid lipid nanoparticles

F.no	Drug(mg)	Glyceryl monostearate (mg)	Tween 40 (mg)	Span 80 (mg)
F1	10	100	10	-
F2	10	150	20	-
F3	10	200	-	10
F4	10	250	-	20

Evaluation of Methotrexate loaded nanoparticles:

Particle size:

To measure the mean particle size and size distribution of Methotrexate -loaded solid lipid nanoparticles (SLNs), which is critical for stability, drug release, and bioavailability.

Zeta Potential:

The zeta potential values of the prepared formulations ranged from -18.4 mV to -25.8 mV. Negative zeta potential values indicate good stability of nanoparticles due to electrostatic repulsion between particles, which prevents aggregation. Among all formulations, F4 showed the highest zeta potential (-25.8 mV) indicating better stability of the nanoparticle system.

SEM analysis:

The surface morphology and shape of Methotrexate -loaded solid lipid nanoparticles were examined using scanning electron microscopy (SEM). A small amount of lyophilized SLN powder was mounted on metallic stubs using double-sided conductive adhesive tape and coated with a thin layer of gold to enhance conductivity and prevent charging. The samples were then observed under the SEM at appropriate accelerating voltages, and images were captured at various magnifications. SEM analysis revealed that the nanoparticles were predominantly spherical with a smooth surface and uniform distribution, confirming the successful formation of SLNs. These morphological observations were correlated with particle size measurements obtained from dynamic light scattering, providing a comprehensive understanding of the nanoparticle characteristics.

Drug Entrapment efficiency:

The entrapment efficiency of Methotrexate in solid lipid nanoparticles was determined using the indirect method. Briefly, a known volume of SLN dispersion was centrifuged at high speed (e.g., 15,000–20,000 rpm) for 30–60 minutes to separate the free (unentrapped) drug in the supernatant from the nanoparticle pellet. The supernatant was carefully collected, and the concentration of unencapsulated Methotrexate was quantified using a validated UV-Visible spectrophotometric method.

$$\text{Entrapment Efficiency (\%)} = \frac{\text{Amount entrapped}}{\text{Total drug loaded}} \times 100$$

In-vitro drug release studies

The in-vitro release of Methotrexate from solid lipid nanoparticles was evaluated using the diffusion method. Briefly, a known quantity of SLN dispersion containing a predetermined amount of Methotrexate was placed into a pre-soaked dialysis membrane with an appropriate molecular weight cut-off (e.g., 12–14 kDa). The sealed dialysis bag was immersed in a dissolution medium, such as phosphate-buffered saline (PBS, pH 7.4) or simulated body fluid, maintained at 37 ± 0.5°C with continuous stirring at 100 rpm to mimic physiological conditions. At predetermined time intervals (e.g., 1, 2, 4, 6, 8 hours), aliquots of the dissolution medium were withdrawn and replaced with an equal volume of fresh medium to maintain sink conditions. The amount of Methotrexate released was quantified using a validated UV-Visible spectrophotometric method.

Percentage of drug release was determined using the following formula.

$$\text{Perentage drug release} = \frac{\text{Da}}{\text{Dt}} \times 100$$

Where, Dt = Total amount of the drug
Da = The amount of drug released

Stability studies:
The stability of Methotrexate -loaded solid lipid nanoparticles was evaluated to assess the formulation's physical and chemical integrity over time under different storage conditions. SLN samples were stored in tightly sealed containers at refrigerated (2–8°C), room temperature (25°C ± 2°C, 60% ± 5% RH), and accelerated conditions (40°C ± 2°C, 75% ± 5% RH) for a period of 3 months.

RESULTS AND DISCUSSION:

Particle size:
With an increase in lipid concentration, the particle size increased. based on entrapment effectiveness and particle size distribution.

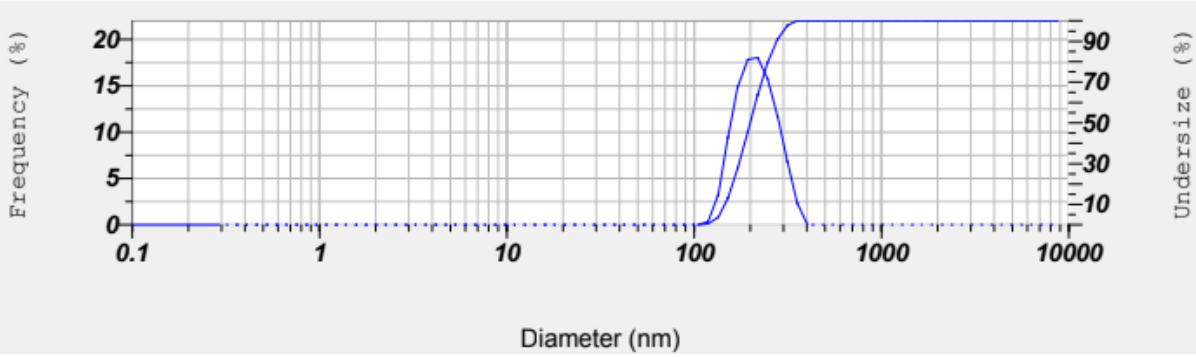


Fig-1: Particle size analysis of Optimized formulation

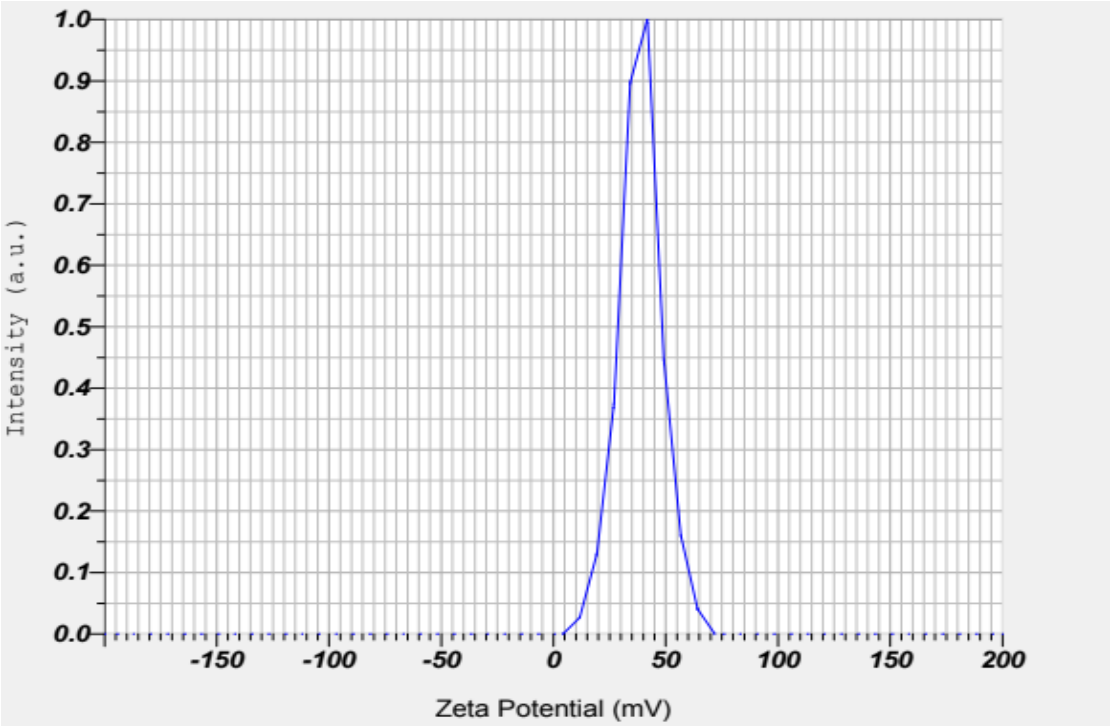


Fig-2: Zeta potential of Optimized formulation

Drug entrapment efficiency:
Optimizing the lipid concentration to be used in the creation of solid lipid nanoparticles was the first step of the work plan. Based on the particle size and entrapment effectiveness of the discovered solid lipid nanoparticles, the lipid content was optimized.

Table-2: Evaluation Studies of Prepared solid lipid nanoparticles: Entrapment Efficiency and Particle size

Batch No	Particle size (nm)	Zeta potential (mV)	Entrapment Efficiency (%)
F1	164	-18.4	72.5
F2	144	-21.6	78.2
F3	121	-23.2	81.4
F4	119	-25.8	85.6

Particle Size:
The particle size of the prepared solid lipid nanoparticles ranged from 119 nm to 164 nm. It was observed that particle size decreased from F1 to F4**,** which indicates better homogenization and stabilization of nanoparticles. Smaller particle size improves drug absorption, stability, and targeting efficiency. Among all formulations, F4 showed the smallest particle size (119 nm) indicating better nanoparticle formation.

Zeta Potential:
The zeta potential values of the prepared formulations ranged from -18.4 mV to -25.8 mV. Negative zeta potential values indicate good stability of nanoparticles due to electrostatic repulsion between particles, which prevents aggregation. Among all formulations, F4 showed the highest zeta potential (-25.8 mV) indicating better stability of the nanoparticle system.

Entrapment Efficiency (EE):
Entrapment efficiency of the prepared SLNs ranged from 72.5% to 85.6%. The results showed that entrapment efficiency increased from F1 to F4, indicating better drug incorporation within the lipid matrix. Higher entrapment efficiency suggests effective drug loading and reduced drug loss during formulation. The highest entrapment efficiency was observed in F4 (85.6%), which indicates better drug encapsulation.

Surface morphology:
According to scanning electron microscopy (SEM), the solid lipid nanoparticles were round, smooth, and free of any aggregation.

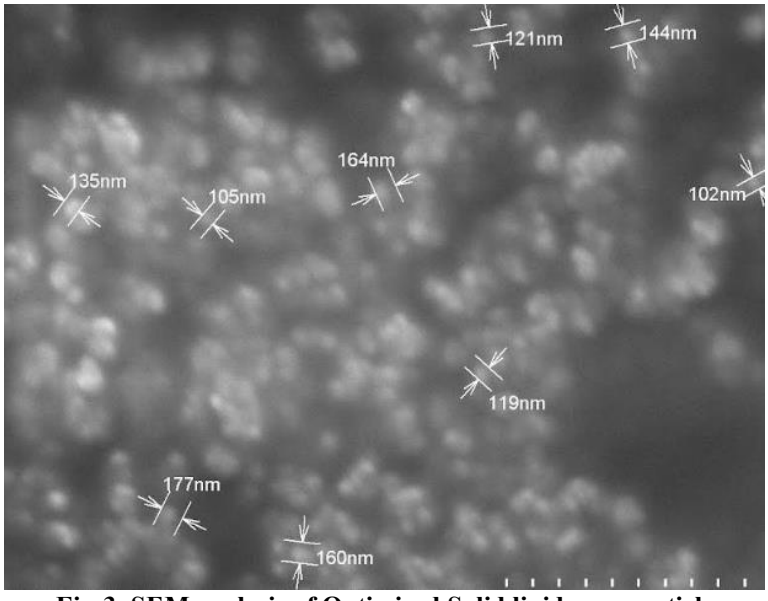


Fig-3: SEM analysis of Optimized Solid lipid nanoparticle

In vitro drug release studies

Table-3: In vitro drug release profiles of SLN (F1-F4)

Time	F1	F2	F3	F4
0	0	0	0	0
1	14.63	15.93	16.83	17.35
2	23.28	25.50	24.58	23.60
3	34.57	35.76	33.21	34.51
4	45.30	44.97	46.58	47.75
5	59.86	66.58	63.28	65.35
6	68.43	74.12	72.15	73.28
7	79.82	81.20	83.25	82.35
8	91.25	93.57	94.55	97.53

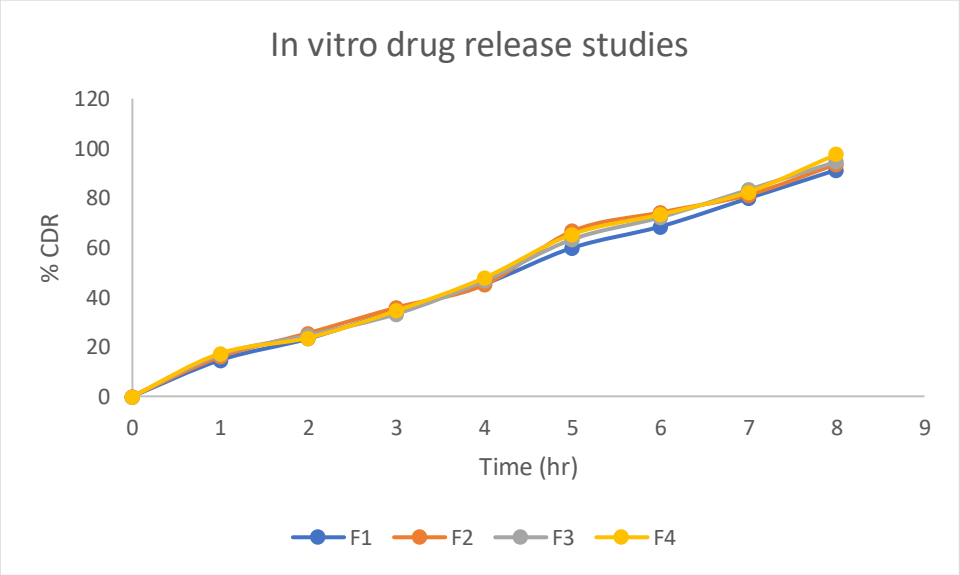


Fig-4: Drug release for (F1-F4) formulations

Observation:
The in-vitro drug release study of SLN formulations (F1–F4) showed a gradual and sustained release pattern over 8 hours. Drug release increased with time for all formulations. Among them, F4 showed the highest cumulative drug release (97.53%), followed by F3 (94.55%), F2 (93.57%), and F1 (91.25%) at 8 hours, indicating better drug release behavior and suggesting F4 as the optimized formulation.

Stability studies:

Table-4: Results of stability studies of optimized formulation F-4

Formulation Code	Parameters	Initial	1st Month	2nd Month	3rd Month	Limits as per Specifications
F-4	**25^{0}C/60%RH**	97.53	96.82	95.74	94.63	**Not less than**
F-4	**30^{0}C/75% RH**	97.53	96.40	95.15	94.84	**Not less than**
F-4	**40^{0}C/75% RH**	97.53	96.96	95.48	94.12	**Not less than**

The stability study results showed that the optimized formulation F4 remained stable for 3 months under different storage conditions, with only a slight decrease in drug content. The values remained within the acceptable limit (NLT 90%), indicating good stability of the SLN formulation.

SUMMARY & CONCLUSION:

Summary:

The present study was focused on the formulation and in vitro evaluation of Methotrexate-loaded Solid Lipid Nanoparticles (SLNs) to improve drug delivery and enhance therapeutic performance. Four SLN formulations (F1–F4) were successfully prepared using glyceryl monostearate as the lipid and Tween 40/Span 80 as surfactants. The FTIR studies confirmed compatibility between Methotrexate and excipients, with no significant drug–excipient interactions observed. Evaluation studies showed that particle size ranged from 119 to 164 nm, with a decrease in size from F1 to F4, indicating improved formulation efficiency. Zeta potential values ranged from -18.4 mV to -25.8 mV, suggesting good colloidal stability. Entrapment efficiency increased from 72.5% to 85.6%, with F4 showing the highest drug loading.

In vitro drug release studies demonstrated sustained release over 8 hours, with formulation F4 showing the highest release (97.53%). Stability studies confirmed that the optimized formulation remained stable under different temperature and humidity conditions over three months with minimal variation in drug content.

CONCLUSION:

Methotrexate-loaded SLNs were successfully formulated and evaluated. The study concluded that SLNs are an effective drug delivery system for improving the stability, entrapment efficiency, and controlled release of Methotrexate. Among all formulations, F4 was identified as the optimized batch based on its smallest particle size, highest entrapment efficiency, highest zeta potential, and maximum drug release. Stability studies further confirmed that the optimized formulation remained stable under various storage conditions. Hence, SLN formulation can be considered a promising approach for enhancing Methotrexate delivery and potentially improving its therapeutic efficacy.

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