FORMULATION AND EVALUATION OF ARSENIC TRIOXIDE SOLID LIPID NANOPARTICLES

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ABSTRACT

The aim of present investigation was the formulation and development solid lipid nanoparticles of Arsenic Trioxide. Study started from the Preformulation study of the drug. While studying IR spectrum, we can conclude that there is no interaction between drug and other excipients. F1-F9 Nine formulations of solid lipid nanoparticles were prepared using soy lecithin, glyceryl tri palmitate, and poloxomer 407. F5 formulation was optimized for its better particle size (152), PDI (0.13), and better zeta potential (-29). Based on F5 formulation, full factorial design was done and formulation was optimized. Factorial batches A1-A9 was prepared using Soya lecithin and Poloxamer 188 as an independent variable. Evaluation of factorial batches was done and found satisfactory. Model found significant for all three responses, Particle Size, Entrapment Efficiency and Polydispersity index (PDI). Validation of design was done and found satisfactory. Based on that optimized batch was selected from the design and evaluated. Optimized batch O1 was charged for stability and found stable for 1 month. Hence, O1 batch was considered as optimized batch.

Key Words: Arsenic Trioxide, lecithin, glyceryl tri palmitate, and poloxomer 407.

1. INTRODUCTION

Introduction of Drug Delivery System

Introduction of Solid Lipid Nano Particles[1]

Solid lipid nanoparticles (SLN) introduced in 1991 represent an alternative carrier system to tradition colloidal carriers such as - emulsions, liposomes and polymeric micro – and nanoparticles. Nanoparticles made from solid lipids are attracting major attention as novel colloidal drug carrier for intravenous applications as they have been proposed as an alternative particulate carrier system. SLN are sub-micron colloidal carriers ranging from 50 to 1000 nm, which are composed of physiological lipid, dispersed in water or in aqueous surfactant solution. SLN

offer unique properties such as small size, large surface area, high drug loading and the interaction of phases at the interface and are attractive for their potential to improve performance of pharmaceuticals.

In order to overcome the disadvantages associated with the liquid state of the oil droplets, the liquid lipid was replaced by a solid lipid, which eventually transformed into solid lipid nanoparticles. The reasons for the increasing interest in lipid-based system are many – fold and include.

1. Lipids enhance oral bioavailability and reduce plasma profile variability.
2. Better characterization of lipoid excipients.
3. An improved ability to address the key issues of technology transfer and manufacture scale-up.

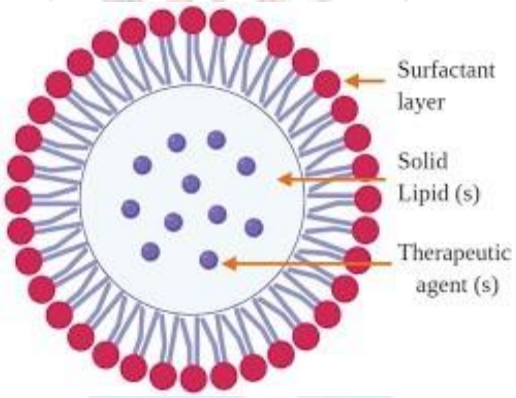

Figure 1 Structure of solid lipid nanoparticle (SLN)

Solid lipid nanoparticles (SLNs) are considered to be the most effective lipid based colloidal carriers, introduced in early nineties. This is the one of the most popular approaches to improve the oral bioavailability of the poorly water-soluble drugs. SLNs are in the submicron size range of 50-1000 nm and are composed of physiologically tolerated lipid components which are in solid state at room temperature.SLNs combine all the advantages of polymeric nanoparticles, fat emulsions and liposomes.

ADVANTAGES OF SOLID LIPID NANO PARTICLE[1-4]

- Control and / or target drug release.
- Improve stability of pharmaceuticals.
- High and enhanced drug content.
- Excellent biocompatibility.
- Raw materials essential the same as in emulsions.
- Enhanced bioavailability of entrapped bioactive compounds.
- Chemical protection of labile incorporated compounds.
- Much easier to manufacture than biopolymeric nanoparticles.
- Better control over release kinetics of encapsulated compounds.
- Easy to scale up and sterilize.

- Very high long-term stability.
- Can be subjected to commercial sterilization procedures.
- Application versatility.

DISADVANTAGES OF SOLID LIPID NANO PARTICLE[4,5]

- Unexpected dynamics of polymeric transitions.
- Unpredictable gelation tendency.
- Particle growth.

AIMS OF SOLID LIPID NANO PARTICLE[5,7]

- Possibility of controlled drug release.
- Increased drug stability.
- High drug pay load.
- No bio-toxicity of the carrier.

METHODS OF PREPARATION OF SOLID LIPID NANOPARTICLES[1-4,5,8,9]

1. High pressure homogenization

A. Hot homogenization

B. Cold homogenization

2. Ultrasonication/high speed homogenization

A. Probe ultrasonication

B. Bath ultrasonication

3. Solvent evaporation method
4. Solvent emulsification-diffusion method
5. Supercritical fluid method
6. Microemulsion based method
7. Spray drying method
8. Double emulsion method
9. Precipitation technique
10. Film-ultrasound dispersion

2. MATERIALS AND METHODS :

2.1 List of Materials

Table 2.1 List of materials

Sr. No.	Material	Role	Sources of Material
1.	Arsenic Trioxide	API	Torrent Research Centre, Ahmedabad
2.	Glyceryl tri palmitate	Lipid Carrier	ACS Chemicals, Ahmedabad.
3	Soya lecithin	Lipid Carrier	ACS Chemicals, Ahmedabad.
4	Poloxamer 407	Polymer	ACS Chemicals, Ahmedabad.

2.2 List of Equipments

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Sr. No.	Equipments	Manufacturers
1.	Digital weighing balance	Reptech weighing balance ltd., Ahmadabad
2.	Overhead Stirrer	Janki Impex Pvt. Ltd, Ahmedabad
3.	Dissolution apparatus	Electro lab ltd, Mumbai
4.	U.V. Visible spectrophotometer	Shimadzu-1601, Kroyoto, Japan.
5.	FTIR	FTIR 8400S, Shimadzu, Kroyoto, Japan.

Pre-Formulation Studies

Preformulation can be defined as investigation of physical and chemical properties of drug substance alone and when combined with excipients.

Preformulation studies are the first step in the rational development of dosage form of a drug substance. The objectives of Preformulation studies are to develop a portfolio of information about the drug substance, so that this information is useful to develop formulation.

Preformulation investigations are designed to identify those physicochemical properties and excipients that may influence the formulation design, method of manufacture, and pharmacokinetic-biopharmaceutical properties of the resulting product. Followings studies performed for in the Preformulation study.

Description

Check the description by visual observation of the API powder and record the observation.

Solubility

Check the solubility of the API by adding the known quantity of the API powder in respective solvent. Calculate the mg/ml and record the results.

Bulk Density:

a) Loose Bulk Density: Weigh accurately 1 g of drug (M), which was previously passed through 20 # sieve and transferred in 100 ml graduated cylinder. Carefully level the powder without compacting, and read the unsettled apparent volume (V_0). Calculate the apparent bulk density in gm/ml by the following formula

Bulk density = Weight of powder / Bulk volume …….. (1)

b) Tapped bulk density: Weigh accurately 1 g of drug, which was previously passed through 20 # sieve and transfer in 100 ml graduated cylinder. Then mechanically tap the cylinder containing the sample by raising the cylinder and allowing it to drop under its own weight using mechanically tapped density tester that provides a fixed drop of 14± 2 mm at a nominal rate of 300 drops per minute. Tap the cylinder for 500 times initially and measure the tapped volume (V_1) to the nearest graduated units, repeat the tapping an additional 750 times and measure the tapped volume (V_2) to the nearest graduated units. If the difference between the two volumes is less than 2% then final the volume (V_2). Calculate the tapped bulk density in gm/ml by the following formula:

Tapped Density = Weight of powder / Tapped volume …….. (2)

Carr's Index

The Compressibility Index of the powder blend was determined by Carr's compressibility index. It is a simple test to evaluate the BD and TD of a powder and the rate at which it packed down. The formula for Carr's Index is as below:

Carr's Index (%) = [(TD-BD) x100]/TD …….. (3)

Hausner's Ratio

The Hausner's ratio is a number that is correlated to the flow ability of a powder or granular material.

Hausner's Ratio = TD / BD ……. (4)

Table 2.3 Effect of Carr's Index and Hausner's Ratio on flow property

Carr's Index (%)	Flow Character	Hausner's Ratio
≤ 10	Excellent	1.00–1.11
11–15	Good	1.12–1.18
16–20	Fair	1.19–1.25
21–25	Passable	1.26–1.34
26–31	Poor	1.35–1.45
32–37	Very poor	1.46–1.59
>38	Very, very poor	>1.60

Angle of repose

The angle of repose of API powder was determined by the funnel method. The accurately weight powder blend were taken in the funnel. The height of the funnel was adjusted in such a way the tip of the funnel just touched the apex of the powder blend. The powder blend was allowed to flow through the funnel freely on to the surface. The diameter of the powder cone was measured and angle of repose was calculated using the following equation.

$$\tan \theta = h/r \quad \text{.....(5)}$$

Where, h and r are the height and radius of the powder cone respectively.

Table 2.4 Effect of Angle of repose (ϕ) on Flow property

Angle of Repose (Φ)	Type of Flow
< 20	Excellent
20-30	Good
30-34	Passable
>35	Very poor

- **Calibration curve of Arsenic Trioxide**

Standard stock solution of drug (100 µg/ml) was prepared by dissolving 10 mg of Arsenic Trioxide in 100 ml using 0.1 N HCl to get a concentration of 100 µg/ml. The prepared solution is sonicated for 10 minutes and filtered through the Whatman No. 41 filter paper. Appropriate volumes of this solution were further diluted to obtain final concentrations in the range of 1 to 10 µg/ml. The spectrum of this solution was recorded from 200 nm to 400 nm using Shimadzu UV-VIS Spectrophotometer.

- **Drug excipients compatibility study**

FTIR studies were carried out to determine the compatibility of excipients with the drug. Pure drug sample and physical mixture of excipients with drug compared by FTIR and check the compatibility.

2.3 Method of Preparation

Hot Homogenizing Method

- The hot homogenizing method of drug-loaded solid lipid particles was prepared.
- Drug 25 mg, solid lipid (50-300mg), and soya lecithin (50-200mg) were dissolved in 10 mL mixture of chloroform and methanol (1:1).
- Organic solvents were completely removed using a rotary evaporator.
- A drug-embedded lipid layer was melted by heating at 5°C above the melting point of the lipid.
- The aqueous phase was prepared by dissolving polaxamer 407 (1.5% w/v) in double distilled water (sufficient to produce 10 mL of preparation) and heated to the same temperature as the oil phase.
- A hot aqueous phase was added to the oil phase, and homogenization was carried out (at 12,000 rpm) using a homogenizer for 10min.
- Coarse hot oil in water emulsion obtained was ultrasonicated using probe sonicator for 20 min.
- Solid lipid nanoparticles were obtained by allowing hot nano emulsion to cool to room temperature.
- The quantity of ingredients used for the preparation of SLNs of Arsenic trioxide is given in below table;

Table 2.5 Formulation table for trial batches F1-F9

Ingredients (mg)	F1	F2	F3	F4	F5	F6	F7	F8	F9
Arsenic Trioxide	25	25	25	25	**25**	25	25	25	25
Glyceryl tri palmitate	100	100	100	-	-	-	-	-	-
Soya lecithin	-	-	-	100	**100**	100	-	-	-
Glyceryl Monostearate	-	-	-	-	-	-	100	100	100
Poloxamer 407	1	-	-	1	-	-	1	-	-
Poloxamer 188	-	1	-	-	**1**	-	-	1	-

Tween 80	-	-	1	-	-	1	-	-	1
Double distilled water (mL)	10	10	10	10	**10**	10	10	10	10
Chloroform: Methanol (1:1) (mL)	10	10	10	10	**10**	10	10	10	10

Table 2.6 Factorial Batches formulation table

Ingredients (mg)	A1	A2	A3	A4	A5	A6	A7	A8	A9
Arsenic Trioxide	25	25	25	25	25	25	25	25	25
Soya lecithin	75	75	75	100	100	100	125	125	125
Poloxamer 188	0.5	1.0	1.5	0.5	1.0	1.5	0.5	1.0	1.5
Double distilled water (mL)	10	10	10	10	10	10	10	10	10
Chloroform: Methanol (1:1) (mL)	10	10	10	10	10	10	10	10	10

2.4 Evaluation of Solid Lipid Nanoparticles of Arsenic Trioxide

- **Particle Sizes, PDI, Zeta Potential:**

The mean particle length and polydispersity index (PDI), that's a degree of the distribution of nanoparticles population, was decided the usage of dynamic light scattering and Zeta capability becomes anticipated on the premise of electrophoretic mobility under an electric powered field, the use of zeta Sizer. Samples were diluted with the distilled water before measurement and measured at a hard and fast angle of 1650 c for the particle size and polydispersity index (PDI) analysis. For the Zeta ability measurement, Samples were diluted as 1:40 ratios with filtered water (v/v) before analysis. Average particle size, PDI and zeta potential have been then measured in triplicate.

- **Drug Content:**

Arsenic trioxide content in solid lipid nanoparticles was assayed by a UV-visible spectrophotometer. Solid lipid nanoparticles (100 mg) were dissolved in 10ml methanol by shaking the mixture for 5 min. One ml of the

resultant solution was taken and diluted to 10 ml with methanol. Then, aliquots were withdrawn, and absorbance was recorded at 285 nm using a UV visible spectrophotometer.

- **Yield of Solid lipid Nanoparticles:**

After complete drying, the solid lipid nanoparticles powders were collected and weighed accurately. The yield of solid lipid nanoparticles was calculated.

- **Entrapment Efficiency:**

Entrapment Efficiency (EE) of the SLNs changed determined by measuring the awareness of uninterrupted drugs in an aqueous medium by centrifugation method. The nanoparticles had been centrifuged during a high-space cooling Centrifuge (C-24. Remi) using nano step centrifuge tubes with ultra-filter out having a relative molecular mass cutoff 100KD (Pall existence sciences-India) at 5000rpm for 15min at 4ºC, and therefore the supernatant was separated. The amount of drug inside the supernatant changed to determining the usage of a UV-Visible spectrophotometer at lambda max 285 nm after suitable dilution. The percent entrapment efficiency (%) changed into calculated using the usage of the subsequent formula:

% EE = Total drug content-Free drug x 100 / Total drug content

- **Percentage of Drug Release**

Franz diffusion cell was used for the in-vitro drug release studies. Semipermeable membrane was placed between donor and receptor chamber of diffusion cell. The receptor chamber was filled with freshly prepared 30 ml 6.8 PH phosphate buffer. SLN equivalent to 100 mg was placed on semi permeable membrane. The franz diffusion cell was placed over a magnetic stirrer (REMI 1ML) with 500 rpm and the temperature was maintained at 37±1°C. 5ml of samples were withdrawn periodically and replaced with fresh buffer. The withdrawn samples were periodically diluted and analyzed for drug content using UV visible spectrophotometer at 285 nm.

- **Accelerated stability study**

Solid Lipid Nanoparticles of Arsenic Trioxide will be kept for one month at accelerated stability conditions (40±2 ºC temperature and 75 ± 5% RH). Samples will be removed and characterized by appearance, drug content and in-vitro drug release study.

3. RESULTS & DISCUSSION

3.1 API Characterization

Description

White to off white crystalline powder.

Table 3.1 Result of Preformulation study of Arsenic Trioxide

Drug	Angle of Repose (^{0})	Loose Bulk Density (g/ml)	Tapped Bulk Density (g/ml)	Carr's Index (%)	Hausner's Ratio
Arsenic Trioxide	27.34	0.375	0.516	27.32	1.376

Conclusion:

From the Results of Preformulation studies of the API, it was concluded that Arsenic Trioxide has poor flow property and compressibility property. But the solid lipid nanoparticles formulation does not require any flow property.

3.2 Drug Excipient Compatibility Study

The Results of compatibility study were given in below table. There is no any change observed in initial and physical mixture sample.

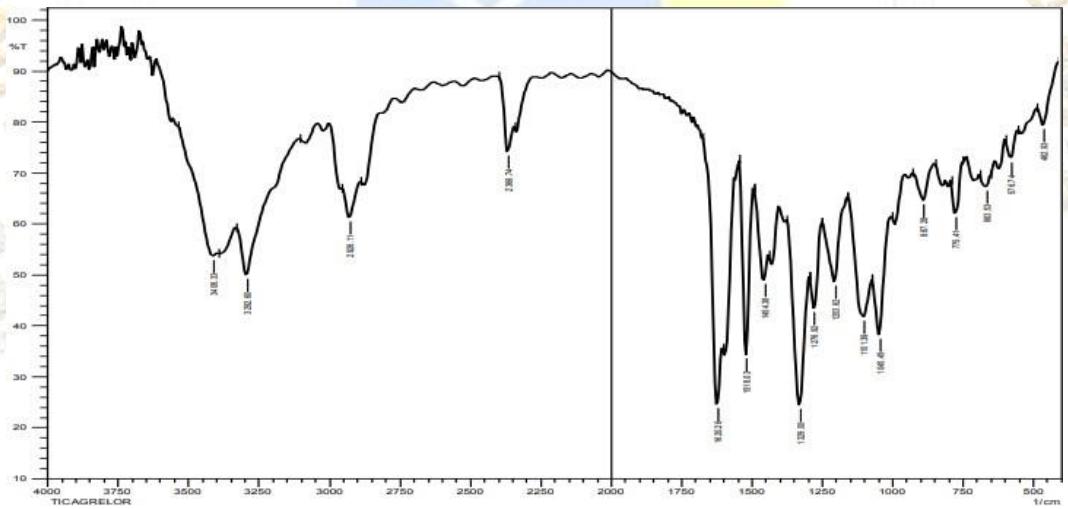

Figure 2 FTIR Spectra of Pure Drug Arsenic Trioxide

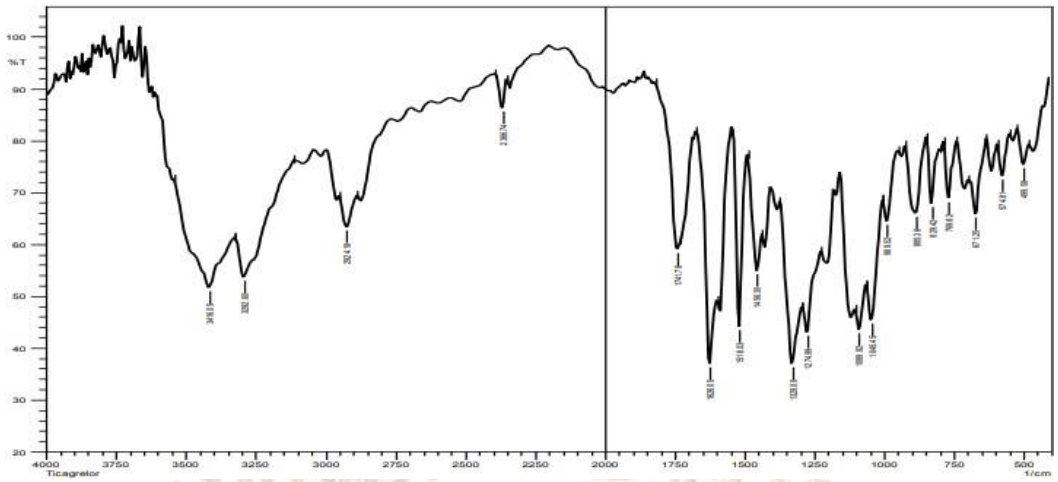

Figure 3 FTIR Spectra of Physical Mixture

Table 3.2 FTIR Data interpretation

Stretching	Pure Drug Peak (cm^{-1})	Formulation Peak (cm^{-1})
C=O	1737	1735
N-H	1629.88	1632.44
C-N	1290.37	1290.12

Conclusion:- From the above FTIR Study, it was concluded that there was no significant Drug-Excipient interaction was observed. So we can conclude that drug and other excipients are compatible which each other.

3.3 Calibration curve of Arsenic Trioxide

The calibration curve of Arsenic Trioxide was found to over a concentration range 1-10 µg/ml. (R^2=0.9998) the data for calibration curve is given below.

Table 3.3 Calibration curve of Arsenic Trioxide

Sr. No	Concentration (µg/ml)	0.1 N HCl
		Absorbance ± SD (n=3)
1	0	0
2	1	0.073 ± 0.005
3	2	0.142 ± 0.004
4	3	0.219 ± 0.003
5	4	0.290 ± 0.006
6	5	0.360 ± 0.004

7	6	0.425 ± 0.003
8	7	0.499 ± 0.005
9	8	0.562 ± 0.001
10	9	0.640 ± 0.006
11	10	0.723 ± 0.002

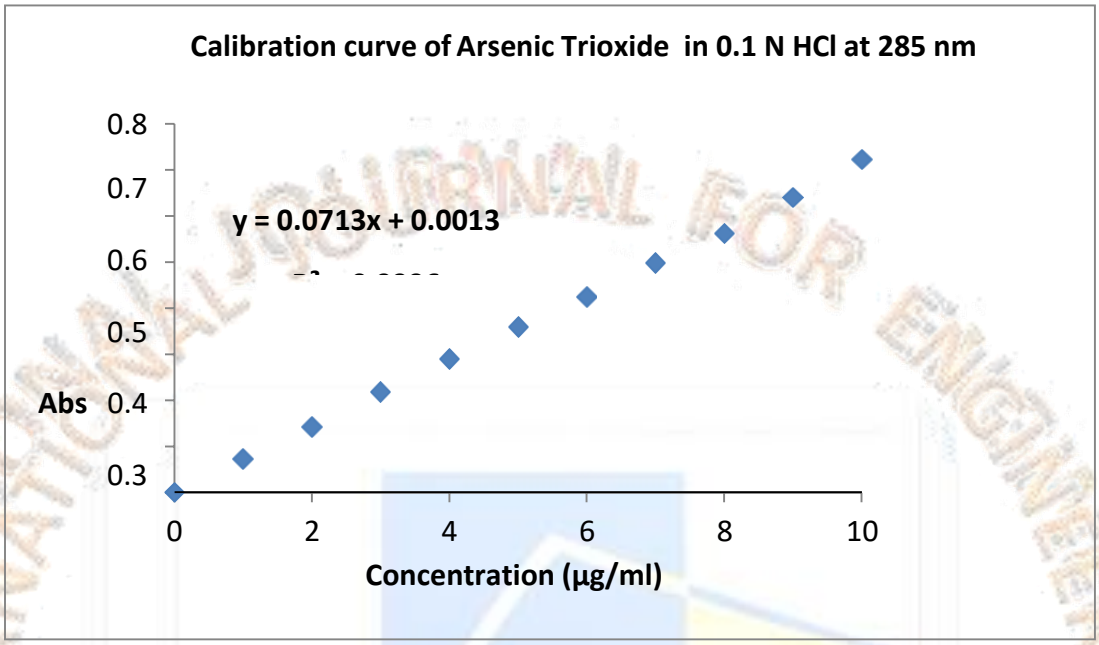

Figure 4 Calibration curve of Arsenic Trioxide in 0.1 N HCl at 285 nm

3.4 Evaluation of Solid Lipid Nano-particles

In present investigation attempt was made to prepare solid lipid nanoparticle of arsenic trioxide. Trial batches F1-F9 was prepared and evaluated for various parameters. The results are given below;

Table 3.1 Evaluation of Solid Lipid Nano-particles

Batch	% Yield	Drug content (%)	Entrapment Efficiency
F1	90.12 ± 0.12	98.52 ± 0.23	89.12 ± 0.08
F2	95.96 ± 0.17	99.26 ± 0.27	94.23 ± 0.09
F3	93.50 ± 0.19	99.45 ± 0.17	92.70 ± 0.06
F4	92.70 ± 0.08	99.67 ± 0.14	90.05 ± 0.07
F5	99.08 ± 0.09	97.62 ± 0.25	98.54 ± 0.03

F6	94.12 ± 0.12	98.27 ± 0.19	89.48 ± 0.05
F7	93.10 ± 0.18	99.15 ± 0.12	91.20 ± 0.08
F8	97.05 ± 0.09	98.12 ± 0.21	93.50 ± 0.07
F9	98.40 ± 0.07	99.02 ± 0.24	95.80 ± 0.06

From the above table 6.4 results it was concluded that the prepared solid lipid nanoparticles are having good entrapment efficiency. Further the drug content was found well within acceptable range. The Practical yield was also observed satisfactory.

Table 3.2 Evaluation of Solid Lipid Nano-particles

Batch	Particle Size (nm)	PDI	Zeta Potential (mV)
F1	231	0.75	-14.9
F2	161	0.34	-21.5
F3	192	0.20	-17.9
F4	201	0.24	-24.8
F5	152	0.13	-29.1
F6	221	0.36	-13.8
F7	259	0.45	-18.5
F8	243	0.56	-14.7
F9	291	0.68	-24.1

Above table shows that the particle size of solid lipid nanoparticles was directly proportional to the concentration of Soya lecithin. Present investigation suggests that increasing the Soya lecithin concentration in the continuous medium increases the particle size of solid lipid nanoparticles. The particle size distribution and polydispersity index (PDI) of lipid based nanocarriers are important physical characteristics to consider when creating food

grade or pharmaceutical-grade products. F5 formulation has the lowest particle size (152) PDI is a number calculated from a two-parameter fit to the correlation data (the cumulants analysis). Lowest PDI value (0.13) for F5 formulation. More Zeta potential is useful for the good stable product. Hence F5 formulation had high zeta potential (-29.1), and it was considered as a good stable product

% Drug release study of trials batches F1-F9 performed and results recorded in Table 3.6.

Table 3.3 Cumulative % drug release of batches F1 to F9

Time (min)	F1	F2	F3	F4	F5	F6	F7	F8	F9
0	0	0	0	0	0	0	0	0	0
5	44.3	38.2	35.9	30.7	45.9	39.7	37.2	32.9	32.9
10	57.8	52.3	47.8	45.2	59.3	53.4	49.7	47.8	47.8
15	77.2	69.8	65.8	62.8	79.1	72.1	67.3	63.8	63.8
20	88.6	81.4	78.2	74.7	89.3	83.4	79.1	75.8	75.8
30	97.1	92.6	89.7	84.2	98.4	93.9	91.4	85.7	85.7

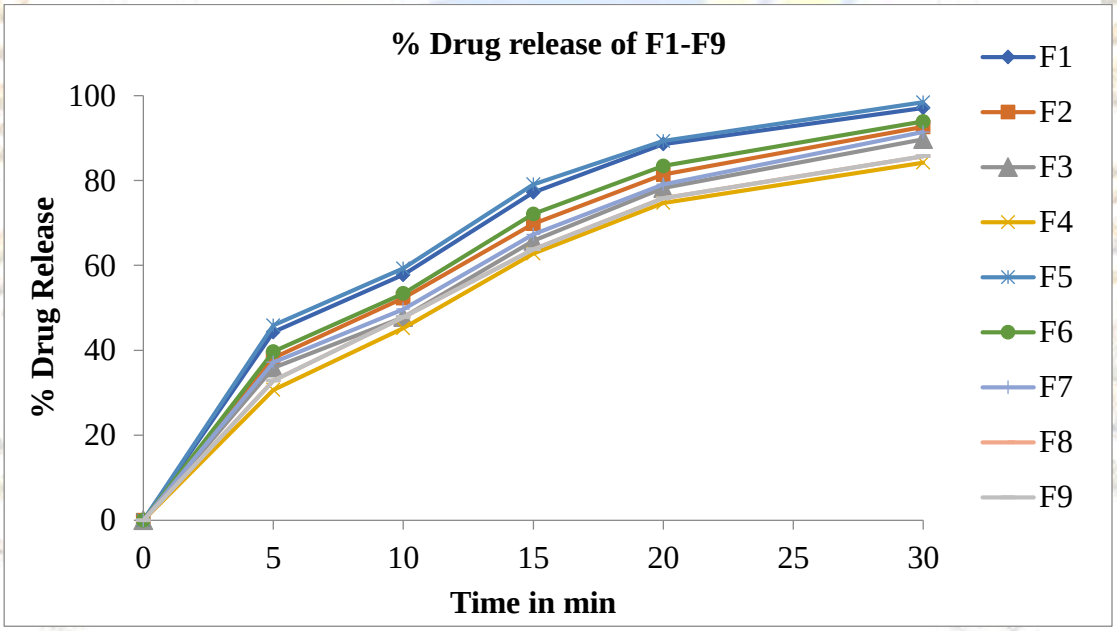

Figure 5 Cumulative % drug release of batches F1 to F9

Table 3.7 Table for Optimized Batch

Sr. No.	Ingredients (mg)	O1
1	Arsenic Trioxide	25
2	Soya Lecithin	110.84
3	Poloxamer 188	0.51
4	Double distilled water (mL)	10
5	Chloroform: Methanol (1:1) (mL)	10

Table 3.8 Evaluation of optimized batch

Evaluation Parameters	Results of O1 Batch
% Yield	89.5 ± 2.5
Drug content (%)	99.6 ± 2.3
Entrapment Efficiency	97.8 ± 2.9
Particle Size (nm)	159 ± 5
PDI	0.14 ± 0.01
Zeta Potential (mV)	-26.5
% Drug Release after 30 mins	99.3 ± 1.8

3.5 Stability Study

Stability study of final optimized batch O1 performed for 1 month at 40°C and 75% RH. Initial results and after 1-month results compared and found satisfactory. The batch was found stable during stability. The results were recorded in below table.

Table 3.9 Stability study of O1 batch

Evaluation Parameters	Initial Results	After 1 Month Results
Appearance	No Change	No Change
Assay (%)	99.6 ± 2.3	99.1 ± 1.5
% Drug Release after 30 mins	99.3 ± 1.8	99.0 ± 1.9

4. CONCLUSION

The aim of present investigation was the formulation and development solid lipid nanoparticles of Arsenic Trioxide. Study started from the Preformulation study of the drug. While studying IR spectrum, we can conclude that there is no interaction between drug and other excipients. F1-F9 Nine formulations of solid lipid nanoparticles were prepared using soy lecithin, glyceryl tri palmitate, and poloxomer 407. F5 formulation was optimized for its better particle size (152),PDI (0.13), and better zeta potential (-29). Based on F5 formulation, full factorial design was done and formulation was optimized. Factorial batches A1-A9 was prepared using Soya lecithin and Poloxamer 188 as an independent variable.Evaluation of factorial batches was done and found satisfactory. Model found significant for all three responses, Particle Size, Entrapment Efficiency and Polydispersity index (PDI). Validation of design was done and found satisfactory. Based on that optimized batch was selected from the design and evaluated. Optimized batch O1 was charged for stability and found stable for 1 month. Hence, O1 batch was considered as optimized batch.

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