RESEARCH ARTICLE

Formulation and Development of Nanoparticulate Drug Delivery System in the Treatment of Cancer

Veerendra Chaurasia, Namita Arora, Pankaj Arora

Lord's University Alwar, Rajasthan

Received: 25th March, 2024; Revised: 10th July, 2024; Accepted: 15th August, 2024; Available Online: 31st August, 2024

ABSTRACT

Cancer is a leading cause of death worldwide. Owing to increased prevalence of cancer globally, it has posed a major challenge to healthcare professionals. The main types of cancer that account for high mortality are lung, stomach, liver, colon, and breast cancer. For many decades and even today, the major portion of chemotherapy is based on intravenous administration of drugs, which has its limitations including lack of safety, inconvenience, and poor patient compliance. In present study, formulated a novel nanoparticulate drug delivery system for the treatment of cancer. Pre- formulation is the initial stage in drug development where the physical and chemical properties of a drug candidate are studied. This phase involves characterizing solubility, stability, pH, and compatibility with excipients to guide the formulation of a safe, effective, and stable dosage form. Pre-formulation studies are crucial for optimizing drug delivery, bioavailability, and shelf-life.

Keywords: Cancer, Pre-formulation and development, Nanoparticles.

International Journal of Pharmaceutical Quality Assurance (2024); DOI: 10.25258/ijpqa.15.3.78

How to cite this article: Chaurasia V, Arora N, Arora P. Formulation and Development of Nanoparticulate Drug Delivery System in the Treatment of Cancer. International Journal of Pharmaceutical Quality Assurance. 2024;15(3):1613-1620.

Source of support: Nil.

Conflict of interest: None

INTRODUCTION

Cancer can be defined as "a cellular malignancy whose unique trait is loss of normal controls resulting in unregulated growth, a lack of differentiation, and an ability to invade local tissues and metastasize".[1,2] These three characteristics: abnormal growth, lack of appropriate differentiation and capacity to invade other tissues or organs are common to different types of cancers. While these characteristics are common in various types of cancers, the extent of each may vary. The approach to treating cancer depends on where in the body it occurs, the type of cells making up the cancer, and how advanced is the cancer.[3,4]

Nanoparticles are solid colloidal particles, ranging in size from 10 to 1000 nm, preferably less than 200 nm. They are made of a macromolecular material, which can be of synthetic or natural origin.[5] Many nanoparticulate carrier systems like emulsions, solid lipid nanoparticles (SLN), polymeric nanoparticles, and liposomes have been developed. Nanoparticles made from solid lipids are attracting increasing attention as colloidal drug carriers for various applications.[6,7] These nanoparticles are in the sub-micron size range and are composed of physiological lipids that are solid at room temperature and form a solid hydrophobic core. They are biodegradable, non-toxic, and involve lower cost of ingredients4. They offer ease of preparation and scale-up, high entrapment of hydrophobic drugs, and possess high dispersibility in an aqueous medium.[8,9]

METHODOLOGY

Pre-formulation study

Differential Scanning Colorimetry (DSC)

A DSC thermogram was obtained in the temperature range of 40-400°C at a 10°C/min rate on the DSC instrument and a scan was obtained.

Ultra Violet Spectrum (UV)

A UV spectrum of the drug was taken at a concentration of 10 mg/mL in methanol from 200 to 400 nm range at room temperature. A 10 mg drug sample was dissolved in a small amount of methanol and the final volume was made up to 250 mL (stock solution) out of this stock solution, 0.1 mL was taken in a 10 mL volumetric flask and diluted to 10 mL by methanol. The spectra were taken in other solvents also like octanol, 0.1N HCl, phosphate buffer (pH 6.8), and distilled water.

Fourier Transform Infra-Red Spectroscopy (FTIR)

An FTIR spectrum was obtained by pellet technique using potassium bromide. A small amount of the drug was mixed with triple the amount of potassium bromide and pellets were made.

Calibration curve of drug sample

The stock solution of methotrexate was prepared by dissolving 25mg of methotrexate in 0.1N sodium hydroxide and the volume was made up to 25ml. The stock solution was diluted with 0.1N sodium hydroxide to obtain the final working

**Author for Correspondence: chaurasiaveerendra@gmail.com*

standards having concentrations of 2, 4, 6, 8, 10, 12, 14, 16, 18 and 20ug/ml. The calibration curve of methotrexate was prepared using the working standard solutions in the range of 2-20ug/ml. The linearity curves of methotrexate were prepared at all the 3 maxima(λmax) observed in the spectrum. Each calibration curve was fitted by linear regression analysis and r2 was calculated. The λmax max having r" closest to 1 was repeated in duplicate.

Solubility studies

- *Solubility*

The solubility of the drug was determined by using the different solvents.

Drug–lipid/emulsifier interaction studies

For investigation of physicochemical changes of pure drug and in combination with additives. A drug interaction study was done. The stability of drugs is affected by the formulation additives. Additives are carefully selected, stable, depend on patient compliance, promote constant release, enhance bioavailability, and protect pharmaceutical actives from degradation. In combinations (drug + lipid) for stability study, these combinations have been stored for a month.

Samples of drugs were weighed and it was mixed with different types of lipids/emulsifiers. These mixers are then put into vials and capped with low-density polyethylene (LDPE). Aluminum caps are used for sealing and closures. These vials are kept under different conditions (5°C), 40°C/75% RH and 40°C for at least four weeks. This vial was observed every week and recorded if there was any physical change. By using the DSC method, the interaction study was performed.

Preparation of Methotrexate and paclitaxel-loaded SLNs (Solid liquid Nanoparticles)

By using ionotropic gelation techniques Methotrexate and paclitaxel-loaded SLN were prepared. By using methanol and chloroform (1:1). Methotrexate, paclitaxel & different types of lipids (stearic acid and compritol) are dissolved. by using a rotary flash evaporator organic phase is been

Formulation Methodology

Microemulsion Technique for the Preparation of SLNs

The microemulsion technique is used for the preparation of Methotrexate and paclitaxel-loaded SLNs. In this technique, lipids were heated at a temperature of 75^0C for the dissolution of drug-molten lipids. Surfactant (poloxamer) and co-surfactant (sodium taurocholate) contained in the aqueous phase were prepared and heated to a temperature of 75^0C. melted lipid phase is been used for the preparation of o/w microemulsion. The temperature of stearic acid (58.97°C) and Compritol ATO (73.42°C) was maintained it was above the melting point. dispersion of microemulsion in ice-cold water under high shear homogenization at the speed of 8000 to 10000 RPM for 15 to 20 minutes with a ratio of 1:16 (v/v). deionized water is used for the washing of ultracentrifuge. After SLNs were diluted in mannitol solution (w/v) before lyophilization.

Figure 1: Solubility Parameter

S.no	*Solvent volume*	*Description*
1.	Very soluble	Less than 1
2.	Freely soluble	From 1 to 10
3.	Soluble	From 10 t0 30
4.	Sparingly soluble	From 30 to 100
5.	Slightly soluble	From 100 to 1000
6.	Insoluble	More than 10000

Lyophilization

30ml of SLNs were placed into a 50ml wide mouth fast–freeze flask. Then these tubes were placed into ultra-low temperature freezer at -20^0C for 12 to 15 hours. these frozen SLNs were lyophilized using a freeze dryer at a temperature -80^0C with 20 to 30mTorr pressure for 24 to 25 hours.

Lipid–Lipid Estimation

Different ratios (1:0, 0:1,1:1,2:1,3:1,4:1,5:1,1:2,1:3,1:4 and 1:5) of stearic acid and compritol were screened. Lipid–lipid ratio is optimized by the smallest particle size mean.

Drug–Lipid Ratio Estimation

For the optimization of the drug-lipid ratio, the drug was weighed and dispersed into a lipid-lipid ratio (1:3) and different drug–lipids ratios of 1:1,1:2,1:3,1:4 and 1:5. A minimum quantity of ethanol is used for the dissolution of poloxamer 188 and sodium taurocholate for the ratio of 1:1(w/w).

Surfactant and Co-Surfactant Ratio Estimation

For surfactants, poloxamer is used and for co-surfactants sodium taurocholate is used. It was decreasing the surfactant uses. It has higher HLB values. It will be required for the emulsifying of the selected lipids. For the optimum HLB values the concentration of both surfactant and co-surfactant was varied from 0.5–1.5% (w/w). For solubilization of poloxamer and sodium taurocholate ethanol is used as a co-solvent. These are non-toxic, polarity, and water miscibility. 2% w/w ethanol concentration, which was used to minimize and soluble the sodium taurocholate. Griffin's formula was used for the determination of HLB values of surfactants with different ratios. HLB (blend) = (% HLB1/100) HLB1 + (% HLB2/100) HLB2 + (% HLB3/100) HLB3

Estimation of Cryoprotectant Concentration

For cryoprotectants, mannitol is used. It may decrease the osmotic activity of water and form the crystallization. It favors the glassy state of the frozen sample. These may prevent the aggregation properties. For stabilization concentration (2.6% to 7.8% (w/v)) of mannitol was used and the parameters were optimized.

Evaluation of Slns Loaded Formulations

Particle size, Zeta potential, Entrapment efficacy

PCS (Photon correlation spectroscopy) is used for the detection of nanoparticles. The polydispersity index was used for the

measurement of subatomic numbers within a polymer. Zeta sizer is used for the determination of particle size, PDI, and zeta potential. By using distilled water lyophilized SLNs were redispersed. These samples were taken into a cuvette and analyzed at 90^0C.

Percentage (%) Yield

30mg of the drug (methotrexate and Paclitaxel) loaded SLNs were transferred into 30 ml of volumetric flasks and 15ml of dimethyl sulphoxide (DMSO) and then sonicated for 30 to 35 minutes. By using DMSO the volume was adjusted by stirring for 15min. after that, this was used for HPLC analysis and the AUC curve was plotted. AUC is used for the measuring of total drug content. The following equations are used:

$$\text{Encapsulation efficiency (\%)} = \frac{\text{total drug} - \text{free drug}}{\text{total drug}} \times 100$$

$$\text{Percentage yield} = \frac{\text{total nanoparticle weight}}{\text{total solid weight}} \times 100$$

$$\text{Loading capacity (\%)} = \frac{\text{drug weight in nanoparticles}}{\text{total weight of nanoparticle}} \times 100$$

Scanning Electron Microscopy (SEM)

SEM (scanning electron microscope) is used for the morphology of SLNs. The samples for scanning electron microscopy were prepared by light sprinkling nanoparticles on a double adhesive carbon tape, which was stuck to an aluminum stub. The stub was then coated with gold to a thickness of 200 to 500 Å under an argon atmosphere using a gold sputter module in a high vacuum evaporator. The samples were then scanned and photomicrographs were taken at 27000x magnifications.

Transmission Electron Microscopy (TEM)

TEM (transmission electron microscopy) is used for the morphology of SLNs. Distilled water with a ratio of 1:10 was used for the dilution of nanogel. One drop of the diluted formulation was subsequently taken and placed onto a carbon-coated copper grid. The excess liquid was removed with filter paper and allowed to stand for 10 m. The grid was then stained with 1% phosphotungstic acid (PTA) and allowed to air dry for 5 m. The sample was then viewed under a transmission electron microscope (TEM) and photomicrographs were taken.

***In vitro* drug release**

This study was performed by the bag diffusion method. This bag membrane should retain the nanoparticle and allow the free drugs to the dissolution media with a cut-off of 15000 molecular weights. Double distilled water is been used for the soaking of this bag. It was remaining in this for 12 to 15 hours before use. 3ml of PBS with pH 6.8 was used for the dispersion of 200mg of lyophilization SLNs. Then this solution was placed into the membrane bag with the two ends fixed by clips. A conical flask is used for the bag placed with the addition of 60ml of PBS pH 6.8. then this the conical flask was fixed on a thermostatic magnetic stirrer with 38^0C at 100RPM. At a certain interval of time, 2 to 3 ml of media were taken out and it was replaced by fresh medium volumes. 0.22 µm is used for the filtration and it was injected by a nylon syringe and assayed by HPLC method.

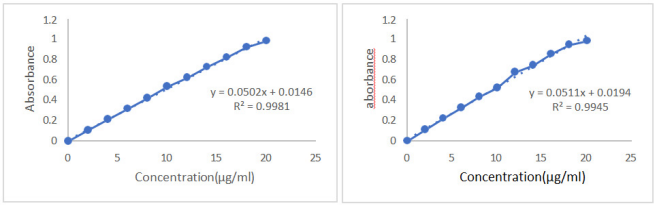

Figure 2: Calibration curve of Methotrexate and Paclitaxel

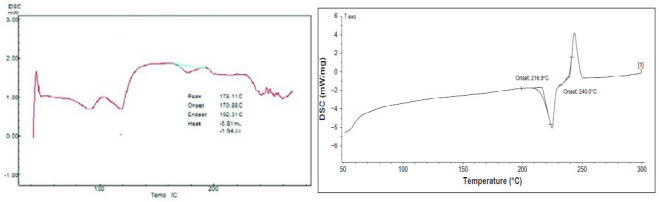

Figure 3: DSC thermogram of Methotrexate and Paclitaxel

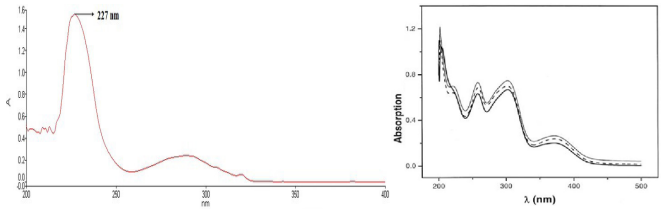

Figure 3: UV Spectroscopy of Paclitaxel and Methotrexate

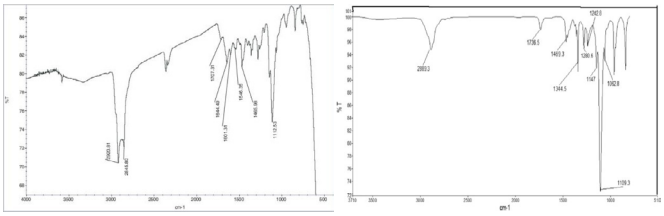

Figure 4: FTIR of Methotrexate and Paclitaxel

RESULTS

Calibration curve

DSC thermogram

Graph showing that the melting point of Methotrexate is 195^0C and Paclitaxel is 216^0C

UV spectroscopy

FTIR study

Solubility Study

These drugs are classified as BCS Class III / IV drugs, which means that they possess low permeability and low solubility. The yellow crystals of methotrexate are practically insoluble in water, ethanol, ether, and chloroform but readily soluble in alkaline hydroxide solutions. The solubility characteristics of methotrexate make its incorporation into lipids extremely challenging to the formulator. Since methotrexate is not readily soluble in the lipid phase, a combination of solubilizers is required to enhance the solubility of the drug in the lipid phase. Solubility studies of methotrexate were conducted in various

solubilizers in combination to increase the solubility and aid the incorporation of the drug into the lipid phase.

Paclitaxel, a highly lipophilic drug, would have more affinity in lipids, and a higher quantity of the drug could be solubilized in the lipid.

Drug–lipid/emulsifier interaction studies

The physical mixture of drugs and excipients (lipid) was examined. This should be kept in different conditions for six weeks. Observation of these vials was done every week. It seemed that there was no change in any physical state compared with the control by using the DSC analyzer, and the interaction study was performed. The pure drug, compritol+ Methotrexate and paclitaxel, stearic acid+ Methotrexate and paclitaxel, and compritol and stearic acid were subjected to DSC evaluation. The melting point of Methotrexate and paclitaxel, stearic acid, and compritol were found to be 210^0C, 70^0C, and 68.5^0C. This study shows the compatibility between Methotrexate and paclitaxel, stearic acid, and compritol.

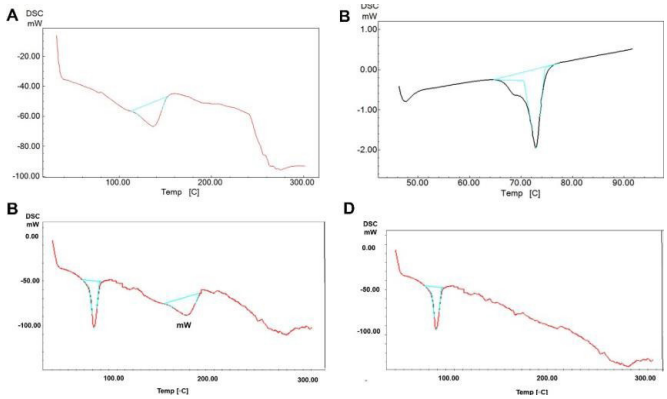

Figure 5: DSC thermograms of (A) methotrexate; (B) Compritol 888; (C) physical mixture of methotrexate and Compritol 888; (D) MTX-SLNs formulation

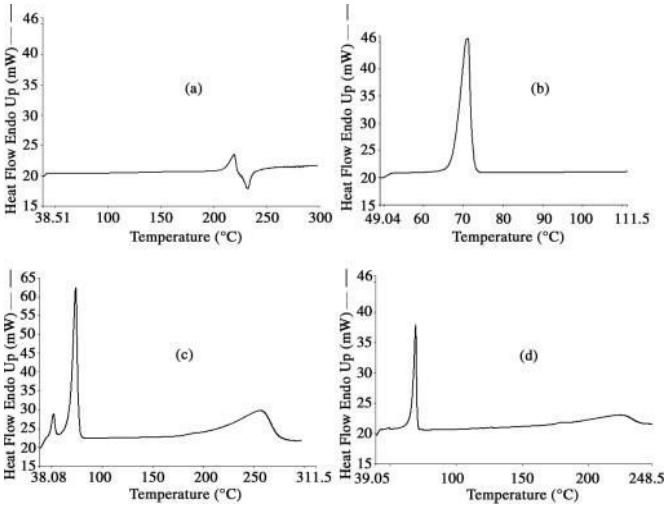

Figure 6: DSC thermogram of (a) paclitaxel, (b) stearic acid, (c) physical mixture, and (d) paclitaxel-loaded stearic acid-SLNs

Formulation of Slns

Different types of lipid proportions were used for the preparation of SLNs. This may act as a biological barrier through diffusion for the release of drugs. The freeze-drying process is used for the termination of SLN preparation. In which DSS are used for stabilizers. The ionotropic gelation technique is used for the preparation of SLNs. The different concentrations (1, 1.6,2,2.5, and 3%) of methotrexate, paclitaxel & lipids are used. Surfactants that are used for the preparations are tween 80, polyethylene glycol, span 20, and at 80^0C temperature, SLN was prepared. This was sufficient to produce 50ml of quantity. Lyophilization is been done at a temperature of -60^0C at 0.020mbar vacuum.

Lipid-lipid ratio estimation

This experiment is performed to determine the factors affecting and factors necessary for the formulation of drug-loaded SLNs. Particle size determination is done in these experiments by the combination of stearic acids and compritol. Using higher partition coefficients lipids were chosen by combining (stearic acid and compritol) in different ratios, and SLNs were formed. Particle size and polydispersity index have been measured. The combination of stearic acids (0.24g) and compritol (0.77) has shown the smallest mean particles. Polydispersity index 385nm and 0.241nm with monomodal size. These may be selected for encapsulation of anti-cancer drugs.

Drug-lipid ratio estimation

The drug-lipid different ratios (1:1 and 1:6) were used for the study of particle size effects. It is been encapsulated in the

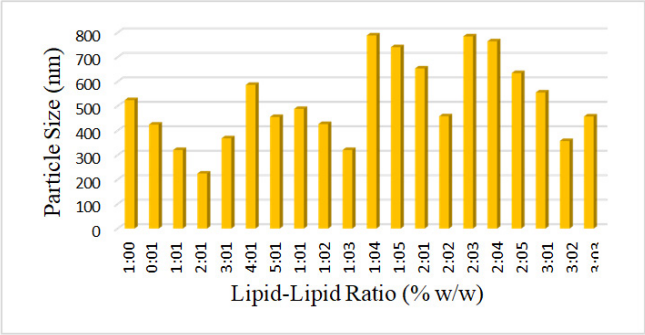

Figure 7: Estimation of lipid–lipid ratio based on particle size of SLNs

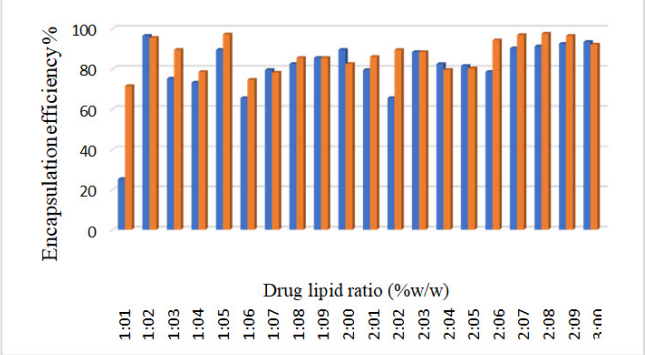

Figure 8: Estimation of Drug-lipid ratio

Table 1: Formulation Of SLN

Formulation code	*Methotrexate, Paclitaxel (% w/v)*	*Lipids (% w/v)*				*Surfactant (%w/v)*
		Glyceryl tri myristate	*DTMAP*	*Glyceryl Mono Stearate*	*Poly-capro lactone*	
F1	1	-	-	-	3.5	T.80 (1%w/v)
F2	1	1	-	-	4.5	T.80 (1.6%w/v)
F3	1	1.5	-	-	0.2	T.80 (2%w/v)
F4	1	2	-	-	0.4	T.80 (2.5%w/v)
F5	1	2.5	-	-	0.6	T.80 (3%w/v)
F6	1	3	-	-	-	PEG (1%w/v)
F7	1	3.5	1	-	-	PEG (1.6%w/v)
F8	1	-	1.5	-	-	PEG (2%w/v)
F9	1	-	2	-	-	PEG (2.5%w/v)
F10	1	-	2.5	-	-	PEG (3%w/v)
F11	1	-	3	1	-	PEG (3.5%w/v)
F12	1	-	3.5	1.5	-	S.20 (1%w/v)
F13	1	-	-	2	-	S.20 (1.6%w/v)
F14	1	-	-	2.5	-	S.20 (2%w/v)
F15	1	1	-	3	-	S.20 (2.5%w/v)
F16	1	1	0.3	3.5	-	S.20 (3%w/v)
F17	1	1	0.4	-	-	S.20 (3.5%w/v)
F18	1	1	0.5	0.2	-	PEG (1%w/v)
F19	1	1	0.6	0.4	-	PEG (1.6%w/v)
F20	1	1	0.7	0.6	-	PEG (2%w/v)

Table 2: Surfactant And Co-Surfactant

Formulation code	*Poloxamer*		*Sodium Taurocholate*		*Ethanol (ml)*	*HLB Value*	*Zeta Potential*	*Avg. Particle*	*PDI*
	(%)	*(g)*	*(%)*	*(g)*			*(mV)*	*size (nm)*	
F1	2	2.58	2	2.15	5	20.29	-39	347	125
F2	2	3.49	2	4.15	5	22.18	-40	329	136
F3	2	4.28	2	6.19	5	23.19	-31	350	140
F4	2	5.78	2	5.17	5	22.08	-25	380	152
F5	2	3.48	2	3.37	5	23.15	-34	390	167
F6	2	2.96	2	4.15	5	21.25	-22	410	175
F7	2	3.88	2	5.19	5	23.17	-35	420	186
F8	2	3.48	2	6.85	5	20.14	-30	450	195
F9	2	2.15	2	4.28	5	22.85	-44	460	210
F10	2	3.17	2	8.29	5	23.18	-51	480	215
F11	2	6.48	2	5.47	5	22.74	-50	521	227
F12	2	4.58	2	1.48	5	20.95	-49	550	234
F13	2	5.55	2	1.74	5	21.85	-53	560	267
F14	2	2.19	2	8.28	5	23.19	-34	570	289
F15	2	3.44	2	6.65	5	20.41	-15	610	295
F16	2	2.45	2	5.65	5	20.15	-16	645	299
F17	2	3.12	2	4.25	5	22.14	-55	712	310
F18	2	6.54	2	1.25	5	23.14	-35	854	352
F19	2	2.21	2	1.35	5	21.62	-26	655	329
F20	2	4.51	2	3.65	5	23.11	-31	553	378

Table 3: Cryoprotectants based on Mean particle size and Polydispersity index

S/no	*Cryoprotectants*	*Mean Particle size*	*Particle size*	*Intensity (%)*	*PDI*
		(nm)	Distribution (nm ± SD)		
1	Fructose	635.8	755.8 ±75.89	68.48	0.489
2	Maltose	540.15	670.6 ± 66.36	98.56	0.647
3	Mannitol	370.5	460.9 ± 75.65	110.5	0.374
4	Sorbitol	399.5	608.4 ± 99.8	88.4	0.456
5	Without Cryoprotectant	360.1	192.3 ± 48.63	75.4	0.357

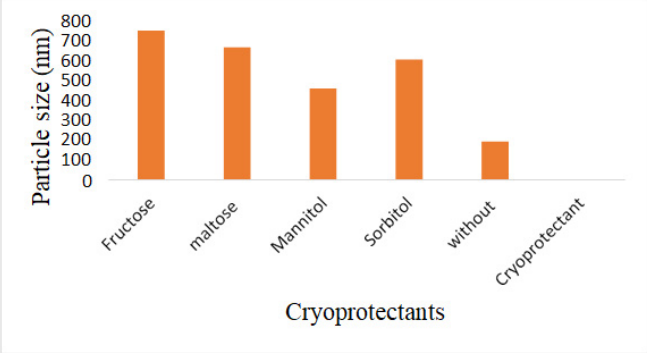

Figure 9: Cryoprotectants based on mean particle size and polydispersity index

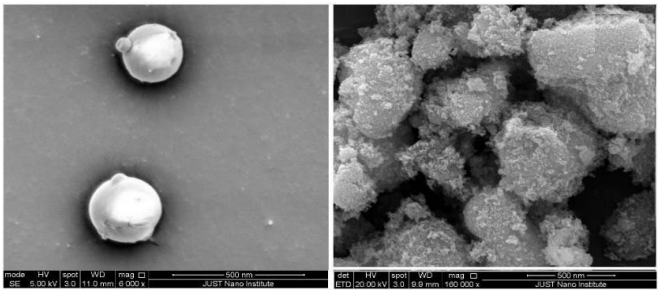

Figure 10: SEM image of MTX-SLNs (A) uncoated SLNs, (B) Coated SLNs with MTXs

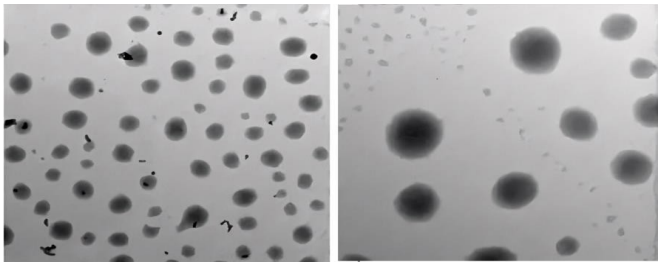

Figure 11: TEM image of MTX-SLNs

SLN's efficiency. Results show that if the concentration of lipids increases it may increase the encapsulation efficiency up to 1:2. After the ratio of 1:2 there were no significant increases in particle size and no encapsulation efficiency was observed

Microemulsion droplets are been surrounded by surfactant and co-surfactant by the interfacial film. In microemulsion, three phases (oil phase, water phase, and inter-phase) are present. SLN preparation by microemulsion technique may be considered in three phases. It may affect the particle size and encapsulation efficiency. This concentration is been increased from 1:1 to 1:6. These may also increase the particle sizes. There were no significant increases in encapsulation efficiency. The drug: lipid was increased up to 1:3. It may also increase the drug lipid ratio. These may not increase the encapsulation efficiency. Due to this saturation of the lipid matrix a higher loading level occurs.

Surfactant And Co-Surfactant Ratio Estimation

Screening of poloxamer and sodium taurocholate with surfactant and co-surfactant may vary the concentration from 1.0 to 1.5%. 3% of co-solvents are kept constant. This may help sodium taurocholate for solubilization.

Concentration is used for the stabilization. It was found that the surfactant and co-surfactant ratio was 22.6. these may be required for the stabilization of the lipids. Formulation F9 shows the lowest particle size (186.48). polydispersity index (PDI) of all the formulations was below (0.700). zeta potential less than -55mV. F9 formulation was used for further optimization.

Estimation Of Cryoprotectant Concentration

Fructose, maltose, mannitol, and sorbitol were used for the cryoprotectants investigation in the lyophilization process for aggregation of SLNs. Redispersion took place in distilled water for the protection of SLN lyophilization with ultrasound for mean particle size analysis. The mean particle size of lyophilized is 1.06 – 12.25 times greater than formulation.

The particle size is larger in fructose as compared to any other substances or without cryoprotectant. Size is been increased from 360.1 to 635.8. most effective cryoprotectants were tested in mean particle size and used for further study.

Evaluation Of Methotrexate Loaded Slns

Particle size analysis, Zeta potential, and PDI

Evaluation of particle range for the formulation. It shows the intention for the finding of the histrionic thickness of particles. The molecule's size was formed in normal ranges (175.2±4.21 to 295.6±3.18nm). these particles were acceptable nanometers. PDI ratio for the mass of the given samples was found to be below 0.5 for SLN formulations. In extent distributions PDI > 0.8. least particle size is been seen in the formulation. Stearic

acid has 0.6%, 4% of compritol and poloxamer, and sodium taurocholate is 4%, and 0.478 PDI shows. SLN formulations show the particle size in range (155.78 to 245.74nm). for pharmaceutical stability, zeta potential has +ve and -ve potential. for good stability, zeta potential has -12 to -21mV agglomeration and zeta potential has -42 to -50mV. Particle size of F6 and F9 has close zeta potential (-50mV).

Scanning Electron Microscopy

SEM was used for the determination of Methotrexate loaded SLNs. SLN will soon be discrete circular using a glossy appearance with no crevices. The image shows that there is a complete removal of the solvent from the formulated SLN, and it also indicates particle size of 200nm that the formulation method was efficient.

Transmission Electron Microscopy

TEM is a plain study the particle morphology by examining the electrons that are granted during the variety. A picture is produced by interpreting the alternation of startling atoms passed through the specimen, which is visualized via an estimating strategy or not precisely away from a rare sensor record.

***In vitro** release studies*

CCD (central composite designed) of *In vitro* drug release of Methotrexate loaded SLNs evaluated by pH 6.9. This was done by the bag diffusion method. Studies show that there was no difference in drug solubility in the buffer. % Cumulative drug release for Methotrexate-loaded SLN formulations ranges from 50.25% to 70% after 24 hours, due to possible degradation of the lower percentage of encapsulated drugs in SLNs. From all the formulations SLN-10 contains 3% stearic acid,4% compritol, and 5% surfactants which has the maximum number of Methotrexate 88.56% in PBS 6.9 after 24 hours.

Evaluation Of Paclitaxel-Loaded Slns

Particle size analysis, Zeta potential, and PDI

Evaluation of particle range for the formulation. It shows the intention for the finding of the histrionic thickness of particles. The molecule's size was formed in normal ranges (176.2±3.25 to 285.7±4.20nm). these particles were acceptable nanometers. PDI ratio for the mass of the given samples was found to be below 0.5 for SLN formulations. In extent distributions PDI > 0.8. least particle size is been seen in the formulation. Stearic acid has 0.7%, 5% of compritol and poloxamer, and sodium taurocholate was 5%, and 0.563 PDI shows. SLN formulations show the particle size in range (168.74 to 268.95nm). for pharmaceutical stability, zeta potential has +ve and-ve potential. for good stability, zeta potential has -13 to -22mV agglomeration and zeta potential has -45 to -51mV. Particle size of F6 and F9 has close zeta potential (-50mV).

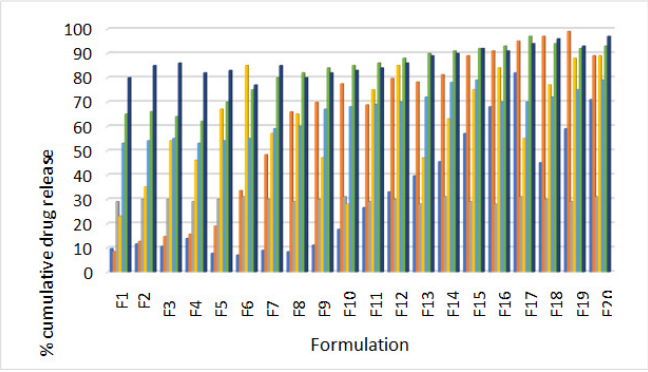

Figure 12: In vitro % CDR of Methotrexate loaded SLN formulations

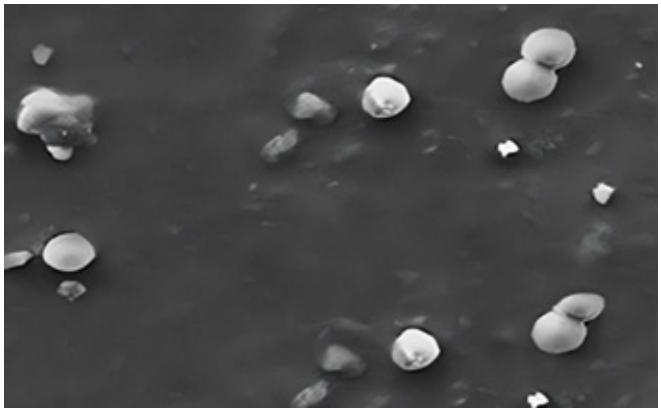

Figure 13: SEM analysis of Paclitaxel

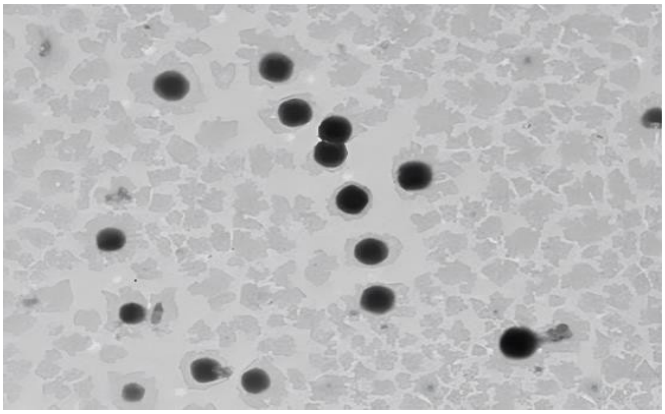

Figure 14: TEM analysis of Paclitaxel-loaded SLNs

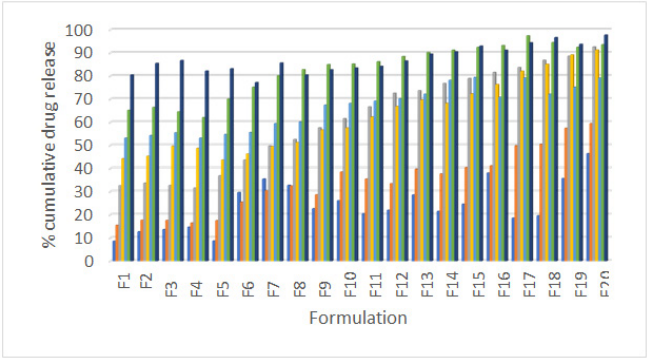

Figure 15: In vitro % CDR of Paclitaxel-loaded SLN formulations

Scanning Electron Microscopy

SEM was used for the determination of Paclitaxel-loaded SLNs. SLN belongs soon to be discrete circular using a glossy appearance with no crevices. The image shows that there is a complete removal of the solvent from the formulated SLN, and it also indicates particle size of 200nm that the formulation method was efficient.

Transmission Electron Microscopy

TEM is a plain study the particle morphology by examining the electrons that are granted during the variety. A picture is produced by interpreting the alternation of startling atoms passed through the specimen, which is visualized via an estimate strategy or not precisely away a rare sensor record.

***In vitro** release studies*

CCD (central composite designed) of *In vitro* drug release of Paclitaxel loaded SLNs evaluated by pH 6.9. this was done by the bag diffusion method. Studies show that there was no difference in drug solubility in the buffer. % Cumulative drug release for Paclitaxel-loaded SLN formulations ranged from 50.25% to 70% after 24 hours. due to possible degradation of the lower percentage of encapsulated drugs in SLNs. From all the formulations SLN-10 contains 3% stearic acid, 4% compritol, and 5% of surfactants which has a maximum number of Paclitaxel 88.56% in PBS 6.9 after 24 hours.

CONCLUSION

Pre-formulation studies were carried out by solubility, melting point, UV spectroscopy, FTIR, DSC, etc. FTIR results showed that there were no drug-excipient interactions. DSC shows that the melting point of Methotrexate is 195^0C and Paclitaxel is 216^0C. The yellow crystals of methotrexate are practically insoluble in water, ethanol, ether, and chloroform but readily soluble in alkaline hydroxide solutions. Paclitaxel being a highly lipophilic drug, would have more affinity in lipids and a higher quantity of drug could be solubilized in the lipid. there was no change in any physical state compared with the control by using the DSC analyzer, and the interaction study was performed. Different types of lipid proportions were used for the preparation of SLNs. This may act as a biological barrier through diffusion for the release of drugs. Evaluation of particle range for the formulation. It shows the intention for the finding of the histrionic thickness of particles. SLN formulations show the particle size in range. SLN belongs soon to be discrete circular using a glossy appearance with no crevices. *In vitro* Studies shows that there was no difference in drug solubility in buffer.

REFERENCES

1. Herbrink M, Nuijen B, Schellens JHM, Beijnen JH. Variability in bioavailability of small molecular tyrosine kinase inhibitors. Cancer Treat Rev. 2015; 41: 412–22.
2. Mazzaferro S, Bouchemal K, Ponchel G. Oral delivery of anticancer drugs I: general
3. considerations. Drug Discovery Today. 2013; 18: 25–34.
4. Banna GL, Collovà E, Gebbia V, Lipari H, Giuffrida P, Cavallaro S. Anticancer oral therapy: emerging related issues. Cancer Treat Rev. 2010; 36: 595–605.
5. Findlay M, von Mankowitz G, Wardley A. Effective oral chemotherapy for breast cancer: pillars of strength. Ann Oncol. 2008; 19: 212– 22.
6. De Jonge ME, Huitema ADR, Schellens JHM, Rodenhuis S, Beijnen JH. Individualized cancer chemotherapy: strategies and performance of prospective studies on therapeutic drug monitoring: a review. Clinical Pharmacokinetic. 2005; 44: 147–73.
7. Kawabata Y, Wada K, Nakatani M, Yamada S, Onoue S. Formulation design for poorly water-soluble drugs based on biopharmaceutics classification system: basic approaches and practical applications. Int J Pharm. 2011; 420: 1– 10.
8. Mazzaferro S, Bouchemal K, Ponchel G. Oral delivery of anticancer drugs III: formulation using drug delivery systems. Drug Discovery Today. 2013; 18: 99–104.
9. Willemsen ACAB, Lubberman FJE, Tol J, Gerritsen WR, van Herpen CML, van Erp NP. Effect of food and acid-reducing agents on the absorption of oral targeted therapies in solid tumors. Drug Discovery Today. 2016; 21: 962–76.
10. Van Leeuwen RWF, Peric R, Hussaarts KGAM, Kienhuis E, Ijzerman NS, de Bruijn P. Influence of the acidic beverage cola on the absorption of erlotinib in patients with non– non-small-cell lung cancer. J Clin Oncol. 2016; 34: 1309–14.