International Journal of Pharmaceutical Research and Applications
Volume 8, Issue 1 Jan-Feb 2023, pp: 1952-1961 www.ijprajournal.com ISSN: 2249-7781

Development and Characterization of Novel Drug Delivery System: To develop and characterize solid lipid nanoparticles (SLN) of the anticancer drug Docetaxel.

Shraddha Parab[1*], Dr. Sudha Rathod [2]

1 Student, M. Pharmacy Pharmaceutics, Oriental College of Pharmacy, Sanpada Navi Mumbai.
2 Principal, Oriental College of Pharmacy, Sanpada Navi Mumbai.
Corresponding Author: Shraddha Parab, Student, M. Pharmacy Pharmaceutics, Oriental College of Pharmacy, Sanpada Navi Mumbai.

Submitted: 05-02-2023 Accepted: 20-02-2023

ABSTRACT : The objective of the study was to develop and characterize a solid lipid nanoparticle (SLN) drug delivery system for delivery of docetaxel. Components of the SLN were lipid (Oleic acid and Soya lecithin) and surfactants (Tween 80). The different batches of docetaxel loaded solid lipid nanoparticles were prepared using different ratios of drug and lipid by solvent emulsification by ultrasonication, size reduction technique. Experiments were carried out with excipients, where surfactants, lipid and drug ratios were varied to optimize the formulation characteristics. The prepared formulations have been evaluated for entrapment efficiency, drug content, in-vitro drug release, particle size analysis, scanning electron microscopy, fourier transform-infrared studies, differential scanning calorimetry and stability.

KEYWORDS : solid lipid nanoparticles, docetaxel, anticancer drug, drug delivery, particle size

I. INTRODUCTION :

Docetaxel is a semi-synthetic antineoplastic agent from the taxoid family which was discovered in 1980s by Pierre Potier from the needles of the western yew tree, Taxus baccata.[1] Docetaxel has been approved by the Food and Drug Administration (FDA) and is widely used for different types of cancer, such as breast cancer, ovarian cancer, prostate cancer, non-small-cell lung cancer, gastric adenocarcinoma, and others.[2] Docetaxel acts by binding reversibly to microtubules, promoting transitory structure stabilization, leading to cell cycle arrest. Therefore, docetaxel is a cytostatic drug for the control of tumor tissue growth.[3]

SLNs were developed in mid 1980s as an alternative system to the existing traditional carriers (emulsions, liposomes, microparticles and their polymeric counterparts) when Speiser prepared the first micro and nanoparticles (named nanopellets) made up of solid lipids for oral administration.[4] SLNs avoid some of their major disadvantages like cytotoxicity of polymers and the lack of a suitable large scale production method for polymeric nanoparticles.[5] SLNs are colloidal carriers made up of lipids that remain solid at room temperature and body temperature and also offer unique properties such as small size (50-500 nm), large surface area, high drug loading and the interaction of phases at the interfaces, and are attractive for their potential to improve performance of pharmaceuticals, nutraceuticals and other materials.[6] Moreover, SLN are less toxic than other nanoparticulate systems because of their biodegradable and biocompatible nature. SLN are capable of encapsulating hydrophobic and hydrophilic drugs, and they also provide protection against chemical, photochemical or oxidative degradation of drugs, as well as the possibility of a sustained release of the incorporated drugs.[7]

Solid lipid nanoparticles formulations can be applied for various routes like parenteral, oral, dermal, ocular, pulmonary, and rectal. The in-vitro and in-vivo studies have shown very positive results.[8]Solid lipid nanoparticles prepared from lipids rather than polymers. The lipids are solid at room temperature. Solid lipid nanoparticles combine the properties of liposomes like biocompatibility and polymeric particle stability, higher production efficiency and the surface solid lipid nanoparticles can be modified for drug targeting by attaching ligands or by PEGylation. They are prepared by homogenization or emulsion precipitation for delivering drugs as a solid molecular dispersion or as a drug encapsulating lipid shell.[9]

The main objective of this study was to develop and characterize solid lipid nanoparticles

International Journal of Pharmaceutical Research and Applications
Volume 8, Issue 1 Jan-Feb 2023, pp: 1952-1961 www.ijprajournal.com ISSN: 2249-7781

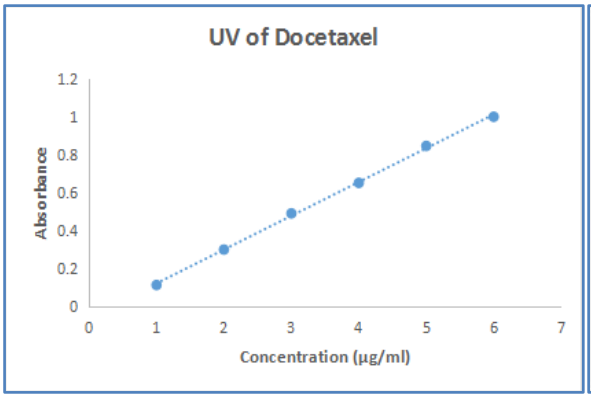
(SLN) of anticancer drug docetaxel. Characterization of docetaxel solid lipid nanoparticles was carried out by different techniques such as fourier-transform infrared spectroscopy (FTIR), particle size analysis, zeta potential, scanning electron microscopy (SEM), and drug release profile.

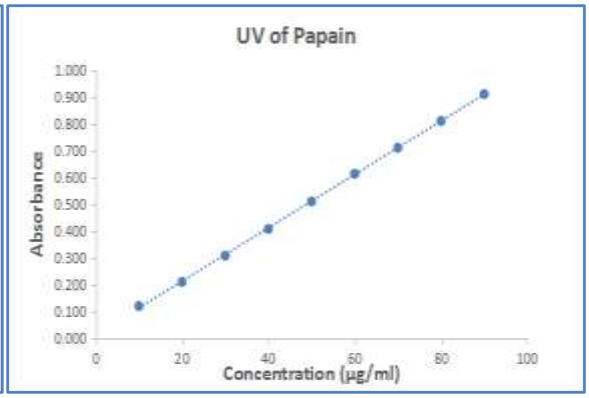
II. MATERIALS AND METHODS :

Docetaxel was obtained from KHANDELWAL LABORATORIES
Oleic Acid from MOLYCHEM and
Soy lecithin from MOLYCHEM
Papain was obtained from PuracBiochem, Netherlands.
All the ingredients were analytical grade. The laboratory grade chemicals used for the work are ethanol, phosphate buffered saline pH 7.4, tween 80, mannitol purchased from HI-MEDIA LABS

III. PREFORMULATION STUDIES

Construction of a calibration curve by ultraviolet (UV) : About 1 mg/ml of docetaxel and papain stock solution was prepared using methanol and further dilutions were made with the same solvent. To a series of 10 ml volumetric flasks, aliquots of standard solutions were taken and the volume was made up to the mark. The absorbance of all the solutions of docetaxel and papain was observed by UV spectrophotometer (UV-1800, Shimadzu, Japan) at 202 nm and 278 nm respectively. A graph was plotted by taking absorbance on Y-axis and concentration on X-axis.





Melting Point : The melting point of the drug and papain was found by capillary method. Docetaxel was found to be in the range of 232 ± 0.5°C and papain was found to be in the range of 29 ± 0.5°C, which was within the limits as per literature. This confirms the purity of the drug and papain.

Solubility : Various solvents such as water, methanol, ethanol and chloroform were used to investigate the maximum solubility of docetaxel and papain. Methanol showed more solubility compared to other solvents.

IV. DESIGN OF FORMULATION

The different batches of docetaxel loaded solid lipid nanoparticles were prepared using different ratios of drug and lipid by solvent emulsification technique which was achieved by ultrasonication, size reduction technique in which ethanol, drug, and lipids constituted the organic phase and phosphate buffer saline pH 7.4, mannitol, tween 80, and Papain constituted the aqueous phase which was further subjected to refrigerated centrifugation. The prepared solid lipid nanoparticles were lyophilized using the Freeze Drying technique.

	Drug : Lipid	Docetaxel (mg)	Oleic Acid (mg)	Soy Lecithin (mg)	Ethanol (ml)	Phosphate Buffer Saline pH 7.4 (ml)	Tween 80 (%)	Papain (mg)	Mannitol (%)

International Journal of Pharmaceutical Research and Applications
Volume 8, Issue 1 Jan-Feb 2023, pp: 1952-1961 www.ijprajournal.com ISSN: 2249-7781

F1	1:3	0	800	400	20	80	3	800	5
F2	1:3	0	600	600	20	80	3	800	5
F3	1:3	0	400	800	20	80	3	800	5
F4	1:4	0	800	800	20	80	3	800	5
F5	1:4	0	1200	400	20	80	3	800	5
F6	1:4	0	400	1200	20	80	3	800	5
F7	1:3	400	800	400	20	80	3	800	5
F8	**1:3**	**400**	**600**	**600**	**20**	**80**	**3**	**800**	**5**
F9	1:3	400	400	800	20	80	3	800	5
F10	1:4	400	800	800	20	80	3	800	5
F11	1:4	400	1200	400	20	80	3	800	5
F12	1:4	400	400	1200	20	80	3	800	5

Method of Preparation of Docetaxel Loaded Solid Lipid Nanoparticles :

The required amount of drug, lipids were weighed as per drug : lipids ratio dissolved in 20 ml ethanol and subjected to Sonication till drug and lipids were completely dissolved. Further phosphate buffer saline pH 7.4 (80ml), tween 80 (3 ml) was added in ethanol mixture and kept for sonication till emulsion was formed. The formulated emulsion was kept in a deep freezer at -18^{0}C for 24 hours. The nanometer size of formulated emulsion was achieved from a 5 hours cycle of bead mill. The nanonized emulsion was centrifuged using refrigerated centrifuge at 5^{0}C to separate supernatant and pellets from which solid lipid nanoparticles were separated, mixed with papain and mannitol. The final solid lipid nanoparticles mixture was further freeze-dried using a freeze dryer for 24 hours cycle at 1 kilopascal vacuum.[10,11,12,13]

- **Selection of organic solvent :** For the selection of organic solvent solubility studies were carried out to find out the solvent in which it has maximum solubility. Various combinations of solvent were investigated by dissolving lipids, drug and herbal extract in it to know the best suited combination of the solvent.
- **Effect of drug lipid ratio :** The following batches were prepared using different drug lipid ratios where aqueous solvent, organic solvent, herbal extract, surfactant and cryoprotectant quantities were kept constant. Then prepared solid lipid nanoparticles were evaluated further to get the desired results in the formulated batches.
- **Effect of centrifugation :** The following batches were centrifuged using refrigerated centrifuge at different speed and time intervals where temperature 5^{0}C was kept constant till a clear supernatant was obtained.

V. CHARACTERIZATION OF SOLID LIPID NANOPARTICLES

1. **pH of the formulation :** The pH of the docetaxel loaded solid lipid nanoparticle formulation was determined using digital pH meter, pH meter 111.
2. **% Practical Yield :** The percentage practical yield is determined to determine the yield or efficiency of any method, which aids in choosing the best production method. The weight of dried SLNs recovered following

International Journal of Pharmaceutical Research and Applications
Volume 8, Issue 1 Jan-Feb 2023, pp: 1952-1961 www.ijprajournal.com ISSN: 2249-7781

lyophilization of an optimized batch was determined as the practical yield as a percentage of the starting ingredients. (Drug + Lipids + Herbal extract + Excipients).[14]

3. **Drug Entrapment Efficiency % and Drug Content % :** The generated solid lipid nanoparticle emulsion was spun for two hours at 20,000 rpm at 50C using a chilled centrifuge. The UV Spectrophotometer 1800 Shimadzu was used to decant the supernatant and evaluate it at 227 nm. Utilizing the formula below, drug entrapment effectiveness and drug content were computed.

Percentage entrapment (%) = $W_{total\ drug\ added} - W_{free\ drug}/W_{total\ drug\ added} \times 100\%$

Drug-loading capacity (%) = $W_{total\ drug\ detected}/W_{total\ solid\ lipid\ added} \times 100\%$

where $W_{total\ drug\ added}$ and $W_{total\ solid\ lipid}$ added were the mass of drug or total solid lipid used for the preparation, respectively,

$W_{free\ drug}$ was the mass of free drug detected in the supernatant after centrifugation of the preparation;

$W_{total\ drug\ detected}$ was the mass of total drug detected in the preparation.[15]

4. **Syringeability :** The syringeability of the formulations was assessed by passing each dispersion through different needle gauges of varying size (18G, 21G, 22G, and 23G). The smallest needle gauge that an entire sample passes through is taken as the syringeability of that sample formulation.[16]
5. **In Vitro Drug Release :** The formulations were tested for drug release in vitro using a dialysis bag. To get rid of any remaining preservative, the membrane was soaked in water for 30 minutes before being connected to the end of the glass tube that housed the donor compartment. A magnetic stir bar was used to move 2 ml of the formulation from the donor compartment into the receptor compartment, which contained 400 ml of phosphate buffer with tween 80 maintained at a temperature of 37^0C and rotating at 300 rpm. The samples were taken out at certain times and a fresh buffer was added right after sampling. These samples were filtered using a 200 nm cellophane membrane filter before being examined spectrophotometrically at 227 nm with the appropriate blank solvent, if necessary.[17]
6. **Batch Optimization :** It was observed that the docetaxel loaded SLN batch (F8) showed highest drug entrapment efficiency, highest drug content, highest production yield, more producibility, high release rate and suitable pH. Therefore, docetaxel loaded SLN batch (F8) was selected for further evaluation studies.
7. **Release Kinetics Studies :** Release data of optimized formulation were fitted to different mathematical models to reveal the release mechanism from the solid lipid nanoparticles : Zero order (% cumulative drug release vs. time)

First order (log % drug release vs. time)

Higuchi model (% cumulative drug release vs. square root of time)

Peppas exponential equation (log % drug release vs. log time)

All curve fitting, simulation and plotting were performed using commercially available Microsoft excel solver and regression coefficient (r^2) values were calculated.[18]

8. **Particle Size Analysis :** Particle size analyzer, Nanophox (NX0073), Sympatec was used to determine particle sizes of docetaxel loaded solid lipid nanoparticle formulations.
9. **Zeta potential :** Zeta potential is an indicator of electrophoretic mobility of particles in dispersion and it was determined by zeta potential analyzer, SZ-100, Horiba.
10. **Fourier transform infrared spectroscopy (FTIR) :** Spectroscopy technique was used in the infrared region to determine the interaction level of functional groups in the docetaxel and final optimized formulation (F8). The changes within the functional group of the sample were observed using fourier transform infrared spectroscopy (IR spirit, shimadzu). FTIR spectra were measured over the range of 4000-400 cm^{-1} with resolution at 4 cm^{-1} for 45 scans.[19]
11. **Differential scanning calorimetry (DSC) :** Thermograms of docetaxel, papain and final optimized batch (F8) were obtained by using differential scanning calorimetry DSC 7020, Hitachi. Samples were weighed, a mass of 3 mg of docetaxel, pappan and final optimized bache (F8) in DSC aluminum crimped pans, and an empty pan were used as reference. DSC was performed at 30-300°C temperature range at the rate of 10°C /min under N_2 flow to provide an inert atmosphere during the

International Journal of Pharmaceutical Research and Applications
Volume 8, Issue 1 Jan-Feb 2023, pp: 1952-1961 www.ijprajournal.com ISSN: 2249-7781

measurement to prevent oxidation reaction.[19]

12. **Particle shape and morphology :** The developed optimized batch of docetaxel loaded solid lipid nanoparticles were characterized for their shape and morphology using scanning electron microscopy (Quanta 200 ESEM, Icon House).

VI. RESULT AND DISCUSSION

Evaluation of Solid Lipid Nanoparticles

The result of Drug Content %, Entrapment Efficiency %, Production Yield%, pH, Drug Release %, and Syringeability (needle) of the formulated batches are shown in table.

Batch	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10	F11	F12
Drug Content	-	-	-	-	-	-	80	85	78	72	70	69
Entrapment Efficiency	-	-	-	-	-	-	82	87	80	75	73	72
% Production Yield	65	62	69	77	70	67	90	94	88	87	85	80
pH	7.51	7.13	7.52	7.33	7.18	7.32	7.23	7.38	7.22	7.55	7.48	7.61
Drug Release (%)	-	-	-	-	-	-	91	96	84	82	77	79
Syringeability	18	18	18	18	18	18	18	18	18	18	18	18

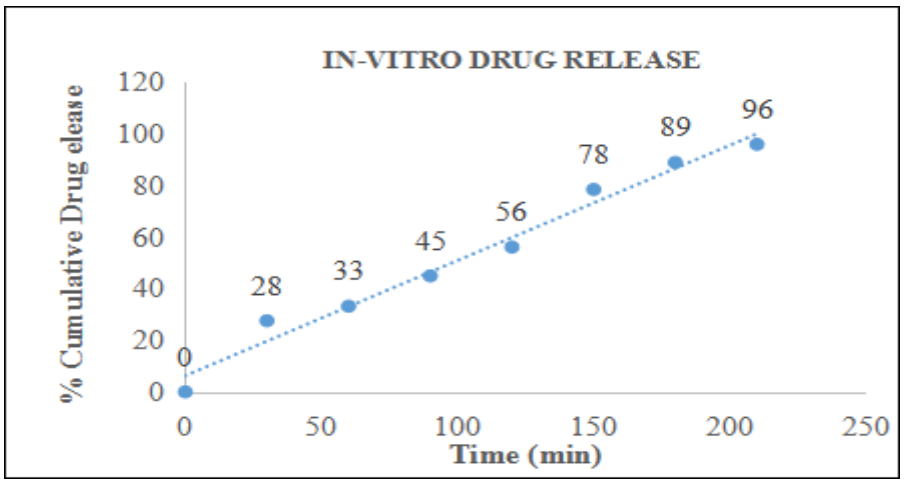
Selection of the Optimized Formulation Batch

The optimized batch was considered to be Batch F8 due to its highest pH (7.38), highest production yield (92 %), highest drug entrapment efficiency (87 %), highest drug content (85 %), more reproducibility, high drug release (96 %), and syringeability (needle 18 gauze) in comparison with other designed batches.

Drug Release Of The Optimized Batch (F8) :

Time (min)	% Drug Release
0	0
30	24
60	33
90	45
120	56
150	78
180	89
210	96

International Journal of Pharmaceutical Research and Applications
Volume 8, Issue 1 Jan-Feb 2023, pp: 1952-1961 www.ijprajournal.com ISSN: 2249-7781



Release Kinetic Studies

The in vitro drug release data of the optimized formula was analyzed for determining the kinetics of drug release. The obtained data were used to find whether the release obeyed Zero order kinetics, First order kinetics, Higuchi or the Korsmeyer-Peppasmodel. The highest correlation coefficient (R^2) obtained from them gives an idea about the model best fitted to the release date. From the results of kinetic studies, the examination of correlation coefficient 'r' indicated that the drug release followed Korsmeyer-Peppas release kinetics. The optimized batch (F8) was subjected to release kinetics studies and the results are as follows:

Release Kinetic Model	Regression Coefficient (R^2)
Zero Order	0.9783
Korsermayer-Peppas	0.9856
Higuchi	0.9864
First Order	0.9899

Kinetic Studies R^2 Values

Particle Size Analysis (PSA) and Polydispersity Index

The particle size of the optimized formulation batch (F8) was found to be 294.43nm and the polydispersity index was found to be 93.07 %.

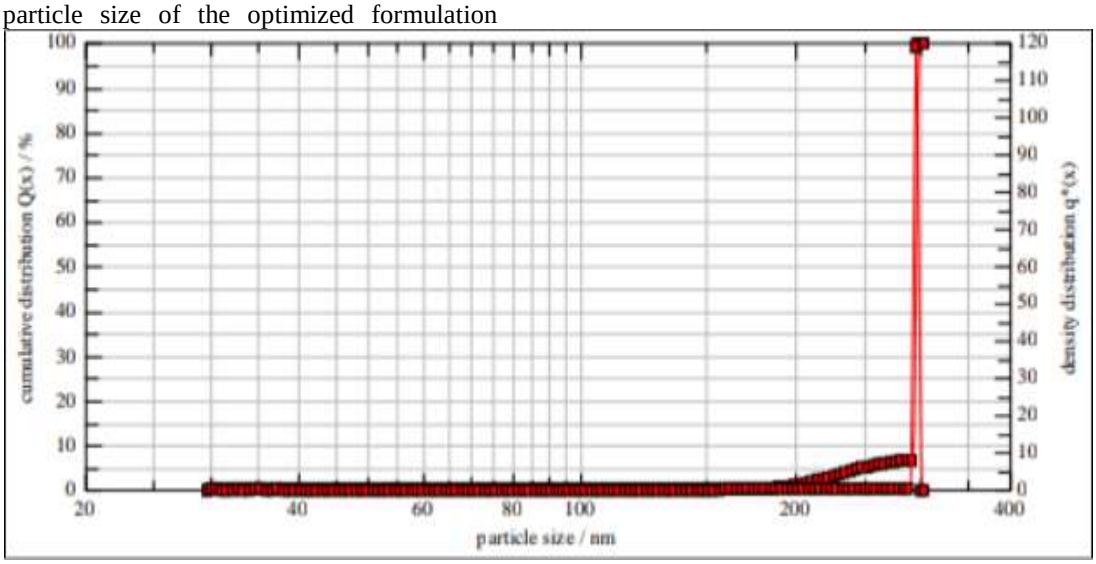


Figure 26 : PSA of Optimized Batch (F8)

International Journal of Pharmaceutical Research and Applications
Volume 8, Issue 1 Jan-Feb 2023, pp: 1952-1961 www.ijprajournal.com ISSN: 2249-7781

Zeta potential

The zeta stability of particles in the dispersion of the optimized formulation batch (F8) was found to be -36.0 mV with electrophoretic Mobility -0.000279 cm^2/Vs which lies in the zeta potential value range of 40mV to -40mV. Hence it confirmed the stability of the formulation.

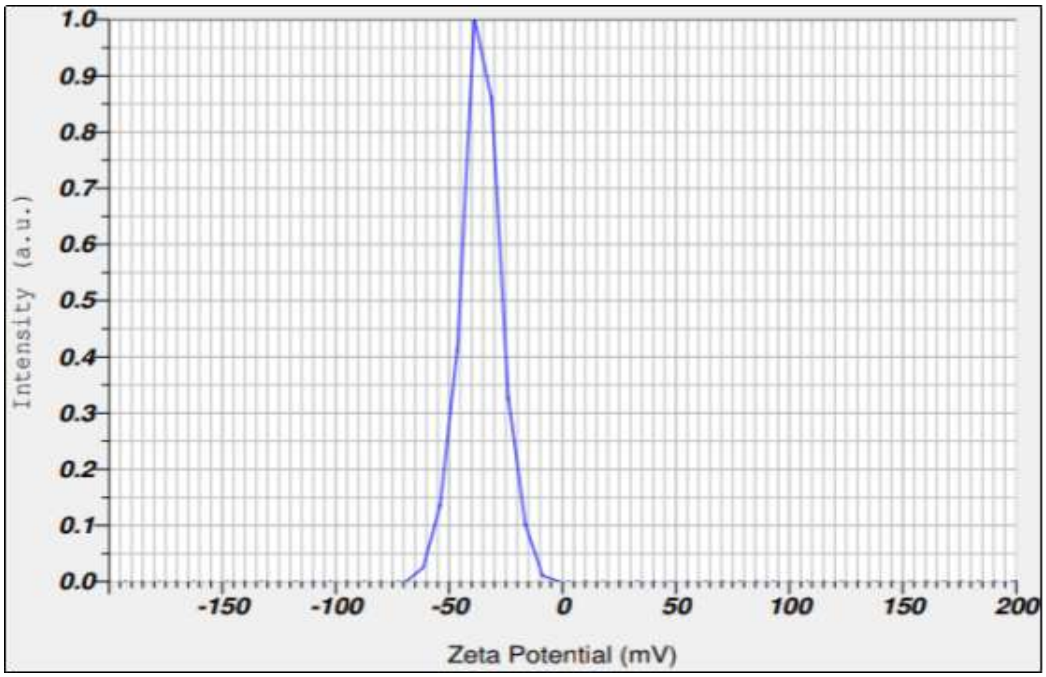
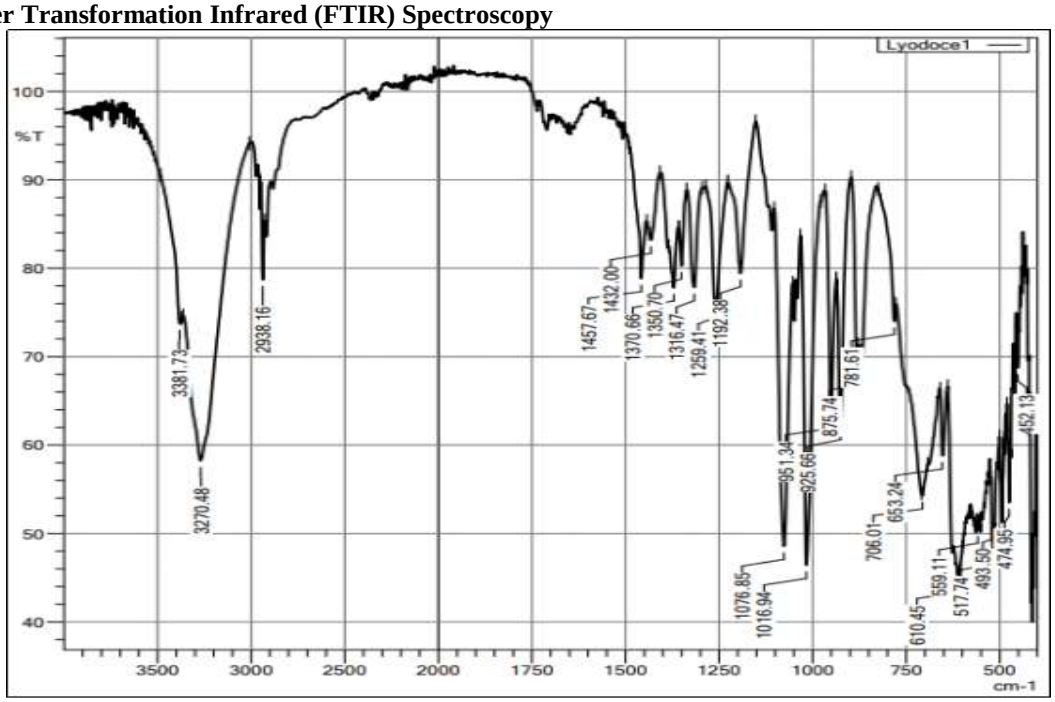


Figure 28 : Zeta potential of Optimized Batch (F8)

Fourier Transformation Infrared (FTIR) Spectroscopy



FTIR of Optimized Batch (F8)

International Journal of Pharmaceutical Research and Applications
Volume 8, Issue 1 Jan-Feb 2023, pp: 1952-1961 www.ijprajournal.com ISSN: 2249-7781

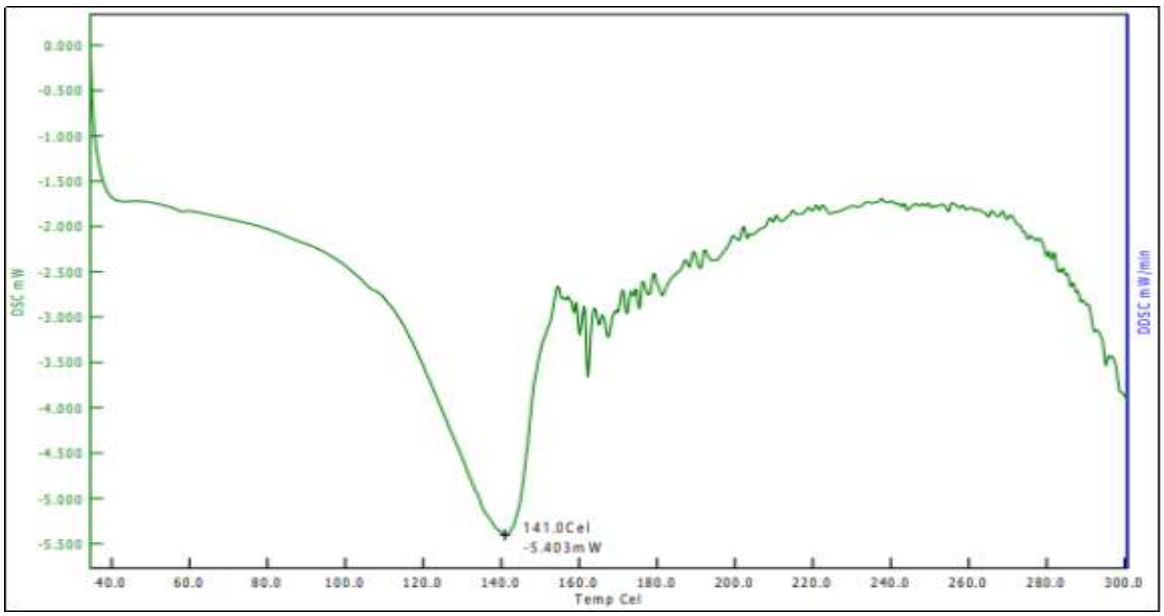
Sr.no.	Peak Range cm^{-1}	Bond and Functional group
1	3270.48	-OH (Hydroxyl group)
2	2938.16	-NH (Amine group)
3	1457.67	Benzene ring
4	1259.41	C-O Ether
5	1076.85	C-O Alcohol
6	875.74	C-H Bending
7	706.01-610.45	C-Cl Alkyl Halide

FTIR of Optimized Batch (F8)

Result: All ingredients of the optimized formulation batch (F8) were found to be compatible with docetaxel after comparing with the standard Fourier-Transform Infrared Spectroscopy (FTIR) of the docetaxel, papain, and other ingredients as there was no significant change in the spectra.

Differential Scanning Calorimetry (DSC)

The Differential Scanning Calorimetry (DSC) of the optimized formulation batch (F8) showed an endothermic reaction at 141^0C.



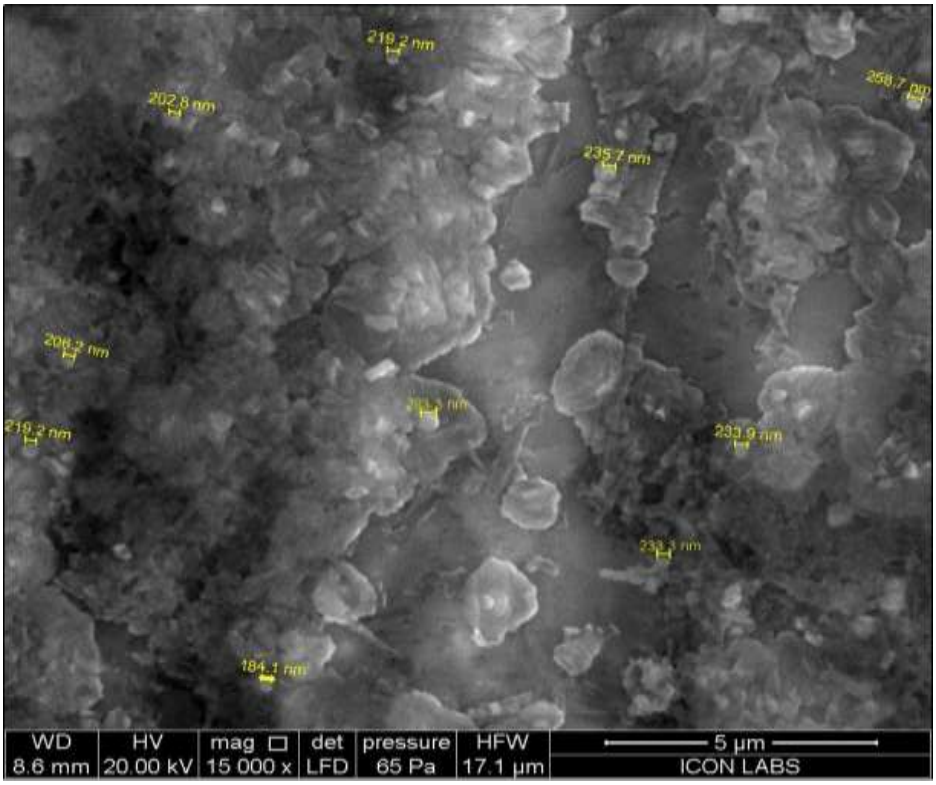
DSC of Optimized Batch (F8)

Result: All ingredients of the optimized formulation batch (F8) were found to be compatible with docetaxel after comparison with the standard Differential Scanning Calorimetry (DSC) of docetaxel, papain, and other ingredients.

Scanning Electron Microscopy (SEM)

The Scanning Electron Microscope (SEM) image of Docetaxel loaded solid lipid nanoparticles shows that the nanoparticles in optimized batch (F8) are spherical in shape and the particle size was found in the range of 180-280 nm.

International Journal of Pharmaceutical Research and Applications
Volume 8, Issue 1 Jan-Feb 2023, pp: 1952-1961 www.ijprajournal.com ISSN: 2249-7781



SEM of Optimized Batch (F8)

Stability Studies

The optimized formulation batch (F8) was found to be stable for 3 months at 5 ± 3^0C and 5 ± 3^0C and 25 ± 2^0C/60 ± 5% RH. The evaluation parameters are shown in the following Table .

Evaluation Parameter	Initial	After 3 Months
Appearance	Off White Powder	Off White Powder
Drug Entrapment Efficiency (%)	87	86
Drug Content (%)	85	84.5
In Vitro Drug Release (%)	96	95

Table no 16: Stability studies of optimized batch

VII. CONCLUSION :

According to the understanding acquired by the extensive literature survey and pharmaceutical background various formulation trials are performed to obtain an optimized formulation. Compatibility studies showed no significant interactions between drug and excipients. The optimized formulation showed satisfactory results in entrapment efficiency as well as in vitro drug release. Optimized formulation subjected to stability studies showed complying results.

REFERENCES

[1]. Anwar A, Ovais M, Khan A, Raza A. Docetaxel-loaded solid lipid

nanoparticles: a novel drug delivery system. IET Nanobiotechnology. 2017 Sep;11(6):621-9.

[2]. Wang L, Liu Z, Liu D, Liu C, Juan Z, Zhang N. Docetaxel-loaded-lipid-based-nanosuspensions (DTX-LNS): preparation, pharmacokinetics, tissue distribution and antitumor activity. International journal of pharmaceutics. 2011 Jul 15;413(1-2):194-201.

[3]. Cho HJ, Park JW, Yoon IS, Kim DD. Surface-modified solid lipid nanoparticles for oral delivery of docetaxel: enhanced intestinal absorption and lymphatic uptake. International journal of nanomedicine. 2014;9:495.

[4]. Speiser P. LipidnanopelletsalsTrägersystem für ArzneimittelzurperoralenAnwendung. European Patent EP. 1990 Aug 8;167825:0167825.

[5]. Smith A. Evaluation of poly (lactic acid) as a biodegradable drug delivery system for parenteral administration. International journal of pharmaceutics. 1986 Jun 1;30(2-3):215-20.

[6]. Cavalli R, Caputo O, Gasco MR. Solid lipospheres of doxorubicin and idarubicin. International journal of pharmaceutics. 1993 Jan 1;89(1):R9-12.

[7]. de Mendoza AE, Campanero MA, Mollinedo F, Blanco-Prieto MJ. Lipid nanomedicines for anticancer drug therapy. Journal of biomedical nanotechnology. 2009 Aug 1;5(4):323-43.

[8]. Mulla JS, Khazi IM, Jamakandi VG. Solid lipid nanoparticles: Potential applications. Indian Journal of Novel Drug delivery. 2010;2(3):82-7.

[9]. Ravi MNV, Handbook of particulate drug delivery, American Sci publishers, California, vol 1, 96-161.

[10]. Gannimitta, Arvind & Srinivas, Prathima & A, Venkateshwar&Pedireddi, Sobhita. (2019). Preparation, Physical Characterization, And Pharmacokinetic Study Of Docetaxel Nanocrystals. Asian Journal Of Pharmaceutical And Clinical Research. 238-244. 10.22159/Ajpcr.2019.V12i6.32856.

[11]. Pooja D, Tunki L, Kulhari H, Reddy BB, Sistla R. Optimization of solid lipid nanoparticles prepared by a single emulsification-solvent evaporation method. Data in brief. 2016 Mar 1;6:15-9.

[12]. Mukherjee S, Ray S, Thakur RS. Solid lipid nanoparticles: a modern formulation approach in drug delivery system. Indian journal of pharmaceutical sciences. 2009 Jul;71(4):349.

[13]. Yang T, Cui FD, Choi MK, Lin H, Chung SJ, Shim CK, Kim DD. Liposome formulation of paclitaxel with enhanced solubility and stability. Drug delivery. 2007 Jan 1;14(5):301-8.

[14]. Nandini PT, Doijad RC, Shivakumar HN, Dandagi PM. Formulation and evaluation of gemcitabine-loaded solid lipid nanoparticles. Drug delivery. 2015 Jul 4;22(5):647-51.

[15]. Zhang JQ, Liu J, Li XL, Jasti BR. Preparation and characterization of solid lipid nanoparticles containing silibinin. Drug delivery. 2007 Jan 1;14(6):381-7.

[16]. Agubata CO, Chime SA, Kenechukwu FC, Nzekwe IT, Onunkwo GC. Formulation and characterization of hydrochlorothiazide solid lipid microparticles based on lipid matrices of Irvingia fat. International Journal of Pharmaceutical Investigation. 2014 Oct;4(4):189.

[17]. https://innovareacademics.in/journals/index.php/ajpcr/article/view/32856

[18]. Sharma M, Kohli S, Dinda A. In-vitro and in-vivo evaluation of repaglinide loaded floating microspheres prepared from different viscosity grades of HPMC polymer. Saudi pharmaceutical journal. 2015 Nov 1;23(6):675-82.

[19]. da Rocha MC, da Silva PB, Radicchi MA, Andrade BY, de Oliveira JV, Venus T, Merker C, Estrela-Lopis I, Longo JP, Báo SN. Docetaxel-loaded solid lipid nanoparticles prevent tumor growth and lung metastasis of 4T1 murine mammary carcinoma cells. Journal of nanobiotechnology. 2020 Dec;18(1):1-20.