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Design and Evaluation of Novel Drug Delivery System: To design and evaluate Paclitaxel-loaded solid lipid nanoparticles

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ABSTRACT: The study's goal was to create and evaluate a Paclitaxel medication delivery system using solid lipid nanoparticles (SLN). Oleic acid and soy lecithin were lipid and surfactant components of the SLN (Tween 80). The several batches of solid lipid nanoparticles containing paclitaxel were made utilizing various dosages of the drug and lipid, solvent emulsification by ultrasonication, and size reduction techniques. Excipients were used in experiments where the ratios of surfactants, lipids, and drugs were changed to improve the formulation properties. Drug content, in-vitro drug release, particle size analysis, scanning electron microscopy, Fourier transform-infrared investigations, differential scanning calorimetry, and stability have all been assessed for the developed formulations.

KEYWORDS: Solid Lipid Nanoparticles, Paclitaxel, Anticancer Drug, Drug Delivery, Particle Size

I. INTRODUCTION:

With more than 10 million new cases each year, cancer continues to be one of the deadliest diseases in the world. However, mortality has dropped in the last two years due to improved diagnostic tools and therapies andstanding of tumor biology. Currently available cancer therapies include surgery, radiation, and chemotherapeutic medications, many of which also cause the patient's healthy cells to die. Therefore, the creation of chemotherapeutics that can either passively or actively target malignant cells would be preferable. Passive targeting takes advantage of the biological characteristics of ttumorsthat cause nanocarriers to build up inside of tumours due to the increased permeability and retention (EPR) effect [1].

Nanoparticles employed as drug delivery vehicles typically have a diameter of less than 100 nanometers in at least one dimension and are made up of biodegradable components such as natural or synthetic polymers, lipids, or metals. Because nanoparticles are more efficiently absorbed by cells than bigger micro molecules, they could be used as effective transport and delivery systems. Drugs can be integrated into the particle matrix or affixed to the particle surface for therapeutic purposes. The fate of a medicine entering the biological environment should be controlled by a drug targeting system. For medication and gene delivery applications, nano systems with various compositions and biological features have been widely explored [2].

Nanoparticles can increase the intracellular concentration of medications in cancer cells while avoiding harm in normal cells utilising passive and active targeting techniques. Furthermore, when nanoparticles bind to certain receptors and enter the cell, they are frequently wrapped by endosomes via receptor-mediated endocytosis, bypassing P-glycoprotein recognition, which is one of the most common drug resistance mechanisms. Although nanoparticles have several advantages as drug carrier systems, they still have a number of drawbacks to overcome, including low oral bioavailability, circulatory instability, insufficient tissue distribution, and toxicity [3].

Nanoparticles, according to the NNI (National Nanotechnology Initiative), are structures with at least one dimension ranging from 1 to 100 nm. The prefix "nano" is widely used for particles with a size of up to a few hundred nano meters. Nanocarriers with optimized physicochemical and biological properties are more easily taken up by cells than bigger molecules, making them viable delivery methods for currently known bioactive chemicals [4].

Nanoparticles are divided into three categories [5]:
Nanoparticles with only one dimension

For decades, a one-dimensional system (thin film or fabricated surfaces) has been used. Thin films (sizes 1–100 nm) or monolayers are already commonplace in the field of solar cells, with applications ranging from chemical and biological sensors to information storage systems, magneto-optic and optical devices, and fibre-optic systems.

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Nanoparticles with two dimensions
Carbon nanotubes (CNTs).
Nanoparticles with three dimensions
Dendrimers, Quantum Dots, Fullerenes (Carbon 60), and other three-dimensional nanoparticles (QDs).

Copper, zinc, titanium, magnesium, gold, alginate, and silver are currently used to make various metallic nanostructures. Nanoparticles are employed for a wide range of applications, including medicinal treatments, use in many industries such as solar and oxide fuel batteries for energy storage, and widespread inclusion into common products such as cosmetics and clothing [6].

For cancer immunotherapy, numerous nanoparticle systems have been investigated. Polymer based nanoparticles are the most common systems among the currently investigated nanoparticles for cancer immunotherapy. Because of their biodegradable, biocompatible, and nontoxic properties, the Food and Drug Administration (FDA) has approved a variety of polymers, including polyethylene glycol, poly (lactide-o-glycolic) acid, and chitosan, for the manufacture of nanoparticle systems for cancer immunotherapy. Inorganic nanoparticles (such as gold nanoparticles) and lipid-based nanoparticles (such as liposomes) are two more extensively employed nanoparticulate systems. All of these nanoparticles have the potential to target cancer and deliver antigens and supplements to the target spot with high precision and accuracy for immune system activation [7].

Solid Lipid Nanoparticles:

The use of solid lipid nanoparticles (SLNs) as a carrier system for water-soluble medication and corrective dynamic treatment. Nanoparticles are colloidal particles with a size between 10 and 1000 nano-meters. They are made up of synthetic polymers and are designed to improve medicine delivery while lowering lethality. They've evolved into a versatile pharmaceutical carrier alternative to liposomes. They are made of manufactured/characteristic polymers and are designed to promote sedate conveyance while reducing lethality. SLN have appealing qualities such as small size, large surface zone, high medicine stacking, and stage communication at the interface, and are enticing for their potential to improve pharmaceutical execution [8].

Solid lipid nanoparticles (SLN) are colloidal nanocarriers with a phospholipid monolayer coating a solid hydrophobic core and a medication encapsulated in high-melting-point glycerides or waxes [9].

Benefits of SLN [10]:

- Drug release control and/or targeting
- Outstanding biocompatibility and enhance medication stability
- Increased and improved drug content
- Better control over encapsulated compound release kinetics
- Entrapped bioactive chemicals have a higher bioavailability
- Labile integrated chemicals are chemically protected

II. INGREDIENTS AND INSTRUMENTS:

A list of ingredients and instruments used in the development of formulation is given below:

Ingredients

Sr. No.	Ingredient	Role/Phase	Grade	Supplied/Gifted/ Prepared By
1	Paclitaxel	Anti-Cancer, Active Pharmaceutical Ingredient	Analytical Grade	Gift sample from Khandelwal Laboratories Ltd
2	Soy Lecithin	Lipid, Internal Phase	Lab Grade	Molychem

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3	Oleic Acid	Lipid, Internal Phase	Lab Grade	Molychem
4	Ethanol	Organic Solvent, Internal Phase	Analytical Grade	Thomas Baker
5	Phosphate Buffer Saline pH 7.4	Aqueous Solvent, External Phase	Lab Grade	Prepared in Oriental College of Pharmacy
6	Tween 80	Surfactant	Lab Grade	RESEARCH-LAB
7	Mannitol	Cryoprotectant	Lab Grade	Himedia Labs
8	Papain	Herbal Extract	Analytical Grade	Omkar Traders

List Of Ingredients

Instruments

Sr. No.	Instrument	Make And Model
1	Electronic Weighing Balance	AI 323, Accuratio India
2	Digital pH Meter	pH meter 111, Pci Analytics India
3	Ultrasonic Cleaner Bath/Sonicator	K.I 1.5, KromTech India
4	Orbital Shaker	LOS-10 LABTOP Instruments Pvt.Ltd. India

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5	Hot Air Oven	Expo hi-tech pvt .Ltd
6	Bead Mill	Rotomate-11, RT-0.2, CD Jay Instruments & Systems Pvt. Ltd. India
7	Refrigerated Centrifuge	BL-150 RS, BIO-LAB India
8	UV-VIS Double Beam Spectrophotometer	UV-1800, Shimadzu Japan
9	Fourier-Transform Infrared Spectroscopy (FTIR)	IRSpirit, QATR-S SINGLE REFLECTION ATR ACCESSORY Shimadzu, Japan
10	Differential Scanning Calorimetry (DSC)	DSC7020, Hitachi, Mumbai
11	Particle Size Analyzer (PSA)	NONOPHOX (NX0073), Cross correlation Sympatic, NANOPHOX CONTROL Mumbai
12	Zeta Potential Analyzer	SZ-100, Horiba, Mumbai
13	Scanning Electron Microscope (SEM)	Quanta 200 ESEM, Icon House, Navi Mumbai
14	Franz Diffusion Cell	EMFDC-06, ORCHID Scientifics, Mumbai India
15	Lyophilizer/Freeze Dryer	LED100-A, LABNICS Instruments, Mumbai
16	Electronic Water Bath	Rajesh chemical, Mumbai
17	Double Distillation Unit	Dolphin, Mumbai India
18	Environment Test Chamber	Dolphin, Mumbai India

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19	Deep Freezer	Vertical Deep Freezer, Microlab Mumbai
20	Magnetic Stirrer	Microlab, Mumbai

List Of Instruments

III. PREFORMULATION STUDIES[11]:

The laboratory investigations to identify the properties of active ingredients and excipients that may affect the formulation, process design, and performance constitute preformulation research. "Learning before doing" has been used to characterize it. The proper design and formulation of dosage forms require consideration of the physical, chemical, and biological characteristics of the drug and excipients used in formulating the product. The drug and excipients must be compatible with one another to produce a product that is stable, efficacious, attractive, easy to administer, and safe. This study was carried out to establish that the therapeutically active drug has not undergone any changes during the formulation of the dosage form.

Authentication Of Drug And Herbal Extract

- **Identification Of Paclitaxel And Papain**

The samples of Paclitaxel and Papain was studied for its organoleptic properties like odour and colour. The result is shown in the following table.

Sr.no	Identification test	Observed result
1	Appearance	White powder
2	Odour	Odourless

Identification Of Paclitaxel

Sr.no	Identification test	Observed Result
1	Appearance	Off white powder
2	Odour	Odourless

Identification Of Papain

Identification Test Of Papain

The identification test of Papain was performed and is as given below:

1 Aqueous Potassium Permanganate Solution + Papain

2 Milk + Papain

Solubility Studies[12]

Based on an immediate-release product's highest dose strength, solubility is determined. Various solvents such as water, methanol, ethanol, and chloroform were used to investigate the maximum solubility of paclitaxel and solvents like water, methanol, ethanol, chloroform, and propylene glycol for papain. Methanol showed more solubility compared to other solvents for both paclitaxel and papain.

UV Spectrum Of Paclitaxel[13]

10mg of Paclitaxel was dissolved in 10 ml of methanol to produce a stock solution of 1000µg/ml. Further 1 ml was withdrawn from the stock solution and diluted to 10 ml with methanol to produce a solution with a concentration of 100µg/ml. 1ml was withdrawn from the 100µg/ml solution and diluted to 10ml using methanol to give a concentration of 10µg/ml which was then scanned in the range of 200-400nm to determine the wavelength of maximum absorbance.

Construction Of Calibration Curve Of Paclitaxel[13]

The calibration curve of Paclitaxel was performed using UV-VIS Double Beam

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Spectrophotometer UV-1800 at λ 227 nm. UV spectrophotometric method for analysis of Paclitaxel was developed in methanol. Paclitaxel was weighed accurately (100mg) and dissolved in methanol in a 100ml volumetric flask. From this stock solution of Paclitaxel 1ml was taken and diluted up to 10ml.

From this solution, 1ml solution was transferred to a 10ml volumetric flask and made up the volume up to 10ml with methanol. The absorbance was recorded at λ 227nm against blank methanol. Again, from the above solution aliquots of 0.4, 0.8, 1.2, 1.6, 2.0, 2.4, and 2.8 ml were pipetted out into 10ml volumetric flask and volume make up sing Methanol to get the concentrations of 4 to 28 µg/ml or ppm. The absorbance of this solution was measured at 227 nm using a UV spectrophotometer against blank methanol.

UV Spectrum Of Papain[13]

10mg of Papain was dissolved in 10 ml of methanol to produce a stock solution of 1000µg/ml. Further 1 ml was withdrawn from the stock solution and diluted to 10 ml with methanol to produce a solution with a concentration of 100µg/ml. 1ml was withdrawn from the 100µg/ml solution and diluted to 10ml using methanol to give a concentration of 10µg/ml which was then scanned in the range of 200-400nm to determine the wavelength of maximum absorbance.

Construction Of Caliberation Curve Of Papain[13]

The calibration curve of Papain was performed using UV-VIS Double Beam Spectrophotometer UV-1800 at λ 278 nm. UV spectrophotometric method for analysis of Papain was developed in methanol. Papain was weighed accurately (100mg) and dissolved in methanol in a 100ml volumetric flask. From this stock solution of Papain 1ml was taken and diluted up to 10ml.

From this solution, 1ml solution was transferred to a 10ml volumetric flask and made up the volume up to 10ml with methanol. The absorbance was recorded at λ 278 nm against blank methanol. Again, from the above solution aliquots of 10, 20, 30, 40, 50, 60, 70, 80, and 90 ml were pipetted out into 10ml volumetric flask and volume makeup using methanol to get the desired concentrations. The absorbance of this solution was measured at 278 nm using a UV spectrophotometer against blank methanol.

Melting Point

The melting point of the Paclitaxel and Papain was found by the capillary method. The capillary filled with powder was placed in Thiele's tube filled with liquid paraffin. The tube was heated and the melting point of the drug powder was noted when it starts to melt.

Fourier-Transform Infrared Spectroscopy (FTIR)[14]

The FTIR spectrum of the drug was obtained using FTIR IRSpirit, QATR-S SINGLE REFLECTION ATR ACCESSORY Shimadzu. FTIR spectra were measured over the range of 4000-400 cm^{-1} with resolution at 4 cm^{-1} for 45 scans. The identity of the drug was confirmed by comparing the IR spectrum of the drug with a reported spectrum of Paclitaxel.

Differential Scanning Calorimetry (DSC)[15]

The Differential Scanning Calorimetry (DSC) of Paclitaxel and Papain was performed using Differential Scanning Calorimetry DSC-7020 Hitachi. Samples were weighed, a mass of 3 mg of docetaxel and papain 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 N2 flow to provide an inert atmosphere during the measurement to prevent oxidation reaction.

Drug Excipients Compatibility Studies With Herbal Extract

In order to comprehend the physical and chemical interactions between the drug and the chosen excipients for the suggested formulation system, the study's goal was to conduct a compatibility study of the two. The samples were kept to test their compatibility in vials wrapped in aluminum foil, or in a closed condition; another batch was kept in vials without foil, or in an open condition, containing a physical mixture of the Paclitaxel, Papain, and other excipients chosen in a ratio of 1:1 and were monitored for any changes over the course of 7, 14, 21, and 28 days.

IV. DESIGN OF FORMULATION:

The different batches of Paclitaxel loaded solid lipid nanoparticles were prepared using different ratios of drug and lipid by solvent emulsification technique which was achieved by ultra-sonication, 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

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aqueous phase which was further subjected to refrigerated centrifugation. The prepared solid lipid nanoparticles were lyophilized using Freeze Drying technique.

The different batches are as follows:

Batch	Drug :Lipid ratio	Drug	Lipid		Tween 80	Ethanol	Phosphate Buffer Saline pH 7.4	Mannitol	Papain
			Soy Lecithin	Oleic Acid					
1	1:3	0 g	0.6 g	1.2 g	3%	20 ml	80 ml	5%	1.2 g
2	1:3	0 g	1.2 g	0.6 g	3%	20 ml	80 ml	5%	1.2 g
3	1:3	0 g	0.8 g	0.8 g	3%	20 ml	80 ml	5%	1.2 g
4	1:3	0.6 g	0.6 g	1.2 g	3%	20 ml	80 ml	5%	1.2 g
5	**1:3**	**0.6 g**	**1.2 g**	**0.6 g**	**3%**	**20 ml**	**80 ml**	**5%**	**1.2 g**
6	1:3	0.6 g	0.8 g	0.8 g	3%	20 ml	80 ml	5%	1.2 g
7	1:4	0 g	0.6 g	1.8 g	3%	20 ml	80 ml	5%	1.2 g
8	1:4	0 g	1.8 g	0.6 g	3%	20 ml	80 ml	5%	1.2 g
9	1:4	0 g	1.2 g	1.2 g	3%	20 ml	80 ml	5%	1.2 g
10	1:4	0.6 g	0.6 g	1.8 g	3%	20 ml	80 ml	5%	1.2 g
11	1:4	0.6 g	1.8 g	0.6 g	3%	20 ml	80 ml	5%	1.2 g
12	1:4	0.6 g	1.2 g	1.2 g	3%	20 ml	80 ml	5%	1.2 g

Trial Batches For Designing Of Formulation

- **Method of preparation of Paclitaxel-loaded solid lipid nanoparticles:**

The required amount of drug and lipids were weighed as per drug:lipid ratio dissolved in 20 ml ethanol and subjected to sonication till drug and lipids were completely dissolved. Further phosphate buffer saline pH 7.4 (80 ml) and Tween 80 (3 ml) was added in the ethanol mixture and kept for sonication till emulsion was formed. The formulated emulsion was kept in Deep Freezer at -18^{0}C for 24 hrs. The nanometre size of formulated emulsion was achieved from the 5 hrs cycle of Bead mill. The nanonized emulsion was centrifuged using Refrigerated Centrifuge at 5^{0}C to separate the supernatant and pellets from which solid lipid nanoparticles were separated, mixed with Papain and mannitol. The final solid lipid nanoparticle mixture was further freeze dried using Freeze Dryer from 24 hrs cycle at 1 Kilopascal vacuum [16,17,18].

- **Selection of organic solvent:**

For the selection of the 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 solvent.

- **Effect of drug lipid ratio:**

The following batches were prepared using different various drug:lipid ratio where aqueous solvent, organic solvent, herbal extract, surfactant and cryoprotectant quantity were kept constant. Then the prepared solid lipid nanoparticles were evaluated further to get the desired results in formulated batches.

- **Effect of centrifugation:**

The following batches were centrifuged using refrigerated centrifuge at different speed and time interval where temperature 5^{0}C was kept constant till clear supernatant was obtained [19].

V. EVALUATION OF SOLID LIPID NANOPARTICLES:

1. Production 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 lyophilization of an optimised batch was determined as the practical yield as a

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percentage of the starting ingredients. (Drug+Lipids+Herbal extract+excipients) [20].
% Production yield= weight of formed solid lipid nanoparticles/Initial weight of Drug+Lipids+Herbal extract+excipients * 100

2. 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 [21].

3. pH of the formulation:

The pH of the Paclitaxel loaded solid lipid nanoparticle formulation was determined using digital pH meter, pH meter 111.

4. Syringeability:

The compositions' syringeability was evaluated by running each dispersion through various needle gauges of differing sizes (18G, 21G, 22G, and 23G). The syringeability of a sample formulation is determined by the smallest needle gauge that a whole sample of solid lipid nanoparticles loaded with paclitaxel can flow through [22].

5. In Vitro Drug Release:

The formulations were tested for drug release in vitro using a dialysis bag. To get rid of any remaining preservatives, 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 370C and rotating at 300 rpm. The samples were taken out at certain times and 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 [23].

6. Batch Optimization:

The batch with the highest drug entrapment efficiency, highest drug content, highest production yield, more reproducibility, high release rate, better syringe ability, and suitable pH was considered to be the optimized batch (Batch 5).

7. Release kinetics studies:

Release data of optimized formulation (Batch 5) 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) and Korsermayer-Peppas exponential equation (log % drug release vs. log time). All curve fitting, simulation and plotting were performed using commercially available Microsoft excel and regression coefficient (R^2) values were calculated [24].

8. Particle Size Analysis:

Particle Size Analyzer (PSA) NONOPHOX (NX0073), Cross-correlation Sympatic, NANOPHOX CONTROL was used to determine the particle size and cumulative distribution in the particle size of the optimized formulation (Batch 5).

9. Zeta Potential:

Zeta Potential Analyzer SZ-100, Horiba was used to determine the zeta potential, the electrophoretic mobility of particles in dispersion of the optimized formulation (Batch 5).

10. Fourier-Transform Infrared Spectroscopy (FTIR):

To ascertain the degree of functional group interaction between Paclitaxel and the improved formulation, spectroscopy was used in the infrared region (Batch 5). Fourier transform-infrared spectroscopy was used to monitor the changes in the functional group of the sample (IR Spirit, Shimadzu). For 45 scans, FTIR spectra in the 4000-400 cm-1 region were obtained with a resolution of at least 4 cm-1 scans [25].

11. Differential Scanning Calorimetry (DSC):

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Using the differential scanning calorimetry DSC-7020 Hitachi, thermograms of Paclitaxel and the improved formulation (Batch 5) were obtained. In DSC aluminum crimped pans, samples containing 3 mg of Paclitaxel and the improved formulation (Batch 5) were weighed. An empty pan served as the standard. In order to maintain an inert atmosphere during the measurement and prevent an oxidation reaction, DSC was carried out in the temperature range of 30 to 300 °C at a rate of 10 °C/min [25].

12. Particle Morphology:

The particle morphology of optimized batch (Batch 5) of Paclitaxel loaded solid lipid nanoparticles was determined using Scanning Electron Microscope (SEM) Quanta 200 ESEM, Icon House.

VI. STABILITY STUDIES:

The optimized formulation (Batch 5) was found to be stable for 3 months at 5±3◦ C/60 ± 5% RH as per ICH guidelines. In the formulation, no physical changes were observed during the stability studies. No significant change in the drug entrapment efficiency, drug content, and in vitro drug release was observed during the stability studies [26].

VII. RESULTS AND DISCUSSION:

1. RESULT FOR AUTHENTICATION OF DRUG AND HERBAL EXTRACT

a. Identification of Paclitaxel and Papain:

The samples of Paclitaxel and Papain was studied for its organoleptic properties like odour and colour. The result are shown in following Table.

Identification test	Observed result	Reported result
Appearance	White powder	White powder
Odour	Odourless	Odourless

Identification Of Paclitaxel

Identification test	Observed Result	Reported Result
Appearance	Off white powder	Off white powder
Odour	Odourless	Odourless

Identification Of Papain

b. Identification Test of Papain:

The identification test of Papain was performed and its result is shown in following Table.

Sr.no.	Identification Test	Result
1	Aqueous Potassium Permanganate Solution + Papain	Decolourises Aqueous Potassium Permanganate Solution
2	Milk + Papain	Curdling Of Milk

Identification Test Of Papain

2. RESULT FOR PREFORMULATION STUDIES:

a. Drug excipients compatibility studies with herbal extract

After 30 days, Paclitaxel and Papain combination with other excipients in a ratio at conditions 30^0C ± 2^0C/65% RH ± 5 % RH and 40^0C ± 2^0C/75% RH ± 5 % RH. Samples were observed for physical changes but there was no physical changes observed in the mixture of Paclitaxel, and Papain combination with other excipients as shown in following Table.

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Batches	Caking	Discoloration	Liquification
Paclitaxel + Soy Lecithin	No Change	No Change	No Change
Paclitaxel + Oleic Acid	No Change	No Change	No Change
Paclitaxel + Ethanol	No Change	No Change	No Change
Paclitaxel + Tween 80	No Change	No Change	No Change
Paclitaxel + Mannitol	No Change	No Change	No Change
Paclitaxel + Papain	No Change	No Change	No Change
Papain + Soy Lecithin	No Change	No Change	No Change
Papain + Oleic Acid	No Change	No Change	No Change
Papain + Ethanol	No Change	No Change	No Change
Papain + Tween 80	No Change	No Change	No Change
Papain + Mannitol	No Change	No Change	No Change

Drug Excipients Compatibility Studies With Herbal Extract

b. Solubility Studies:

Various solvents were used to investigate the maximum solubility of Paclitaxel and Papain. The result is shown in following Table.

Solvent	Solubility (mg/ml)
Methanol	15.3
Water	0.05
Chloroform	10.08
Ethanol	12.5

Solubility Studies Of Paclitaxel

Solvent	Solubility (mg/ml)
Methanol	16.5
Water	12.9
Chloroform	6.2
Propylene glycol	6.8
Ether	2.6

Solubility Studies Of Papain

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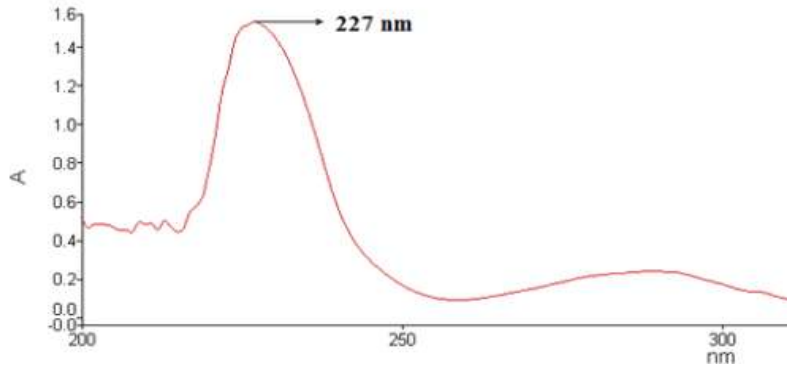
c. UV spectrum of Paclitaxel:

The solution of Paclitaxel in methanol was found to exhibit maximum absorption (λ max) at 227 nm after scanning in a range of 200-400 nm.



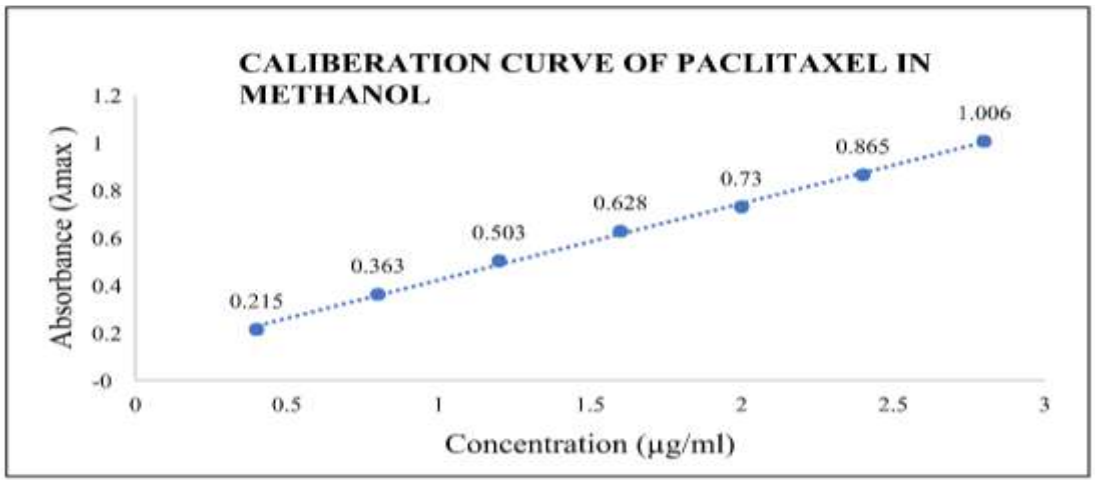
UV Spectrum Of Paclitaxel

d. Construction of caliberation curve of Paclitaxel:

The calibration curve for Paclitaxel drug was determined in Methanol.

Concentration (µg/ml)	Absorbance (λmax =227nm)
0.4 0.8 1.2	0.215 0.363 0.503
1.6	0.628
2 2.4 2.8	0.73 0.865 1.006

Caliberation Curve Of Paclitaxel In Methanol



UV Spectrum Of Paclitaxel

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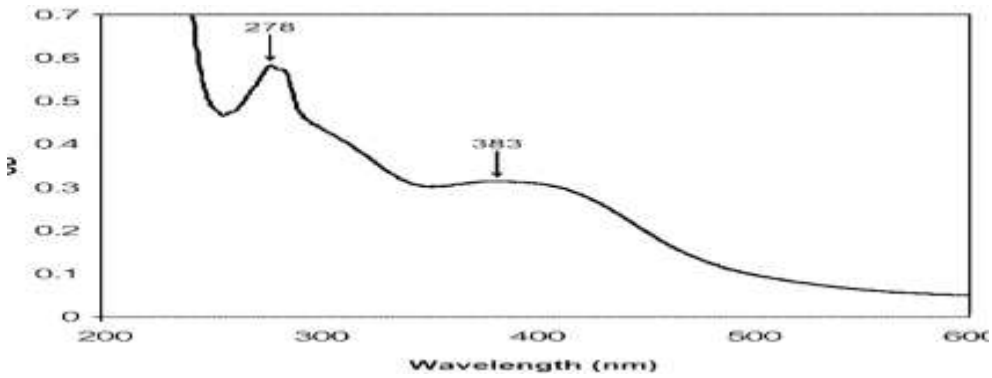
e. UV spectrum of Papain:

The solution of Papain in methanol was found to exhibit maximum absorption (λ max) at 278 nm after scanning in a range of 200-400 nm.



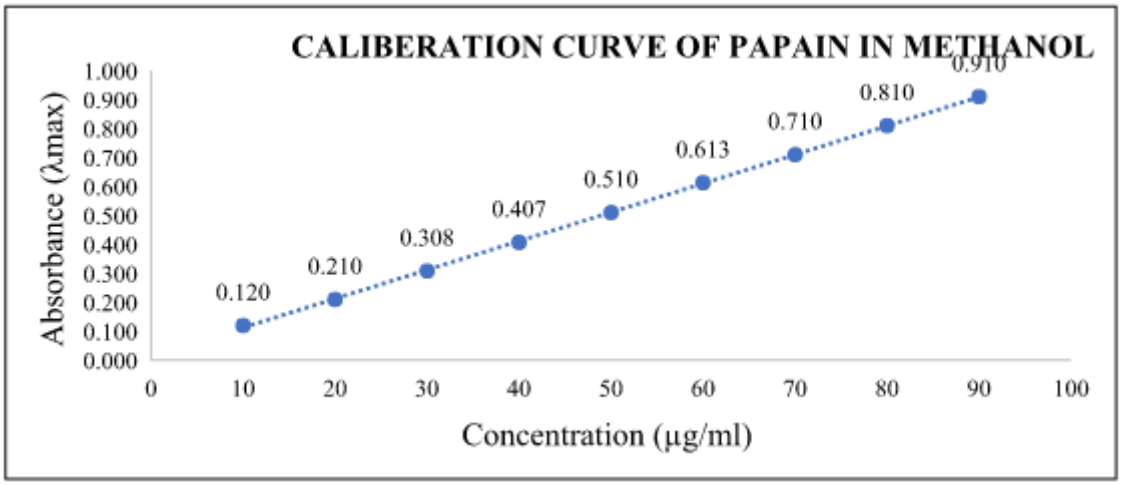
UV Spectrum Of Papain

f. Construction of caliberation curve of Papain

The calibration curve for Papain was determined in Methanol.

Concentration (µg/ml)	Absorbance (λmax =278nm)
10	0.120
20	0.210
30	0.308
40	0.407
50	0.510
60	0.613
70	0.710
80	0.810
90	0.910

Caliberation Curve Of Papain In Methanol



UV Spectrum Of Papain

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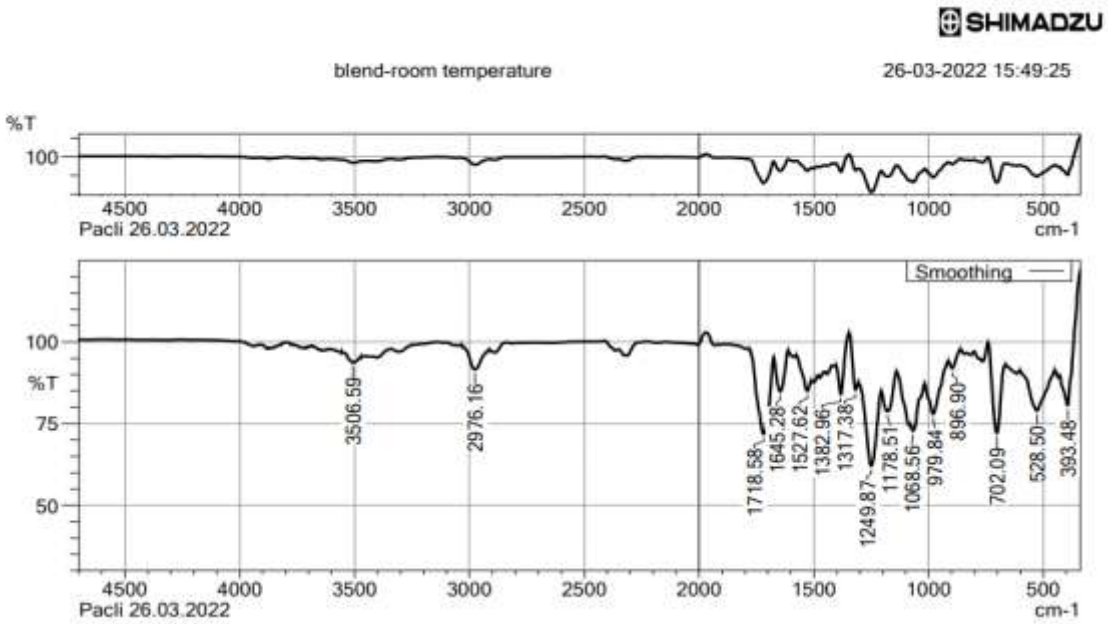
g. Melting Point:

The melting point of Paclitaxel by capillary method was found to be approximately 217 ± 0.5 ^{0} C respectively, which was within the limits as per literature. This confirms the purity of Paclitaxel. The melting point of Papain by capillary method was found to be approximately 29 ± 0.5 ^{0} C respectively, which was within the limits as per literature. This confirms the purity of Papain.

h. Fourier-Transform Infrared Spectroscopy (FTIR):

The identity of drug was confirmed by comparing IR spectrum of drug with reported spectrum of Paclitaxel as shown in Figure and Table gives the functional groups.

The interaction level of functional groups in the Fourier-Transform Infrared Spectroscopy (FTIR) of Paclitaxel is shown in following Table.



Fourier-Transform Infrared Spectroscopy (FTIR) Of Paclitaxel

Sr.no.	Peak Range cm^{-1}	Bond and Functional group
1	528.5, 702.09	Benzene rings
2	896.9, 979.84	=C-H, Alkenes and Aromatic compounds
3	1068.56,1178.51, 1249.87	C-O-C, C-OH Ether Alcohol
4	1317.38, 1382.96	CH3, CH2 Alkanes Alkenes
5	1645.28	-C=C, Aromatics
6	1718.58	-C=O Ester, Ketones
7	2976.16	-CH3, -N-H Aliphatic groups, Amines
8	3506.59	-OH (Hydroxyl group)

Fourier-Transform Infrared Spectroscopy (FTIR) Of Paclitaxel

Result: It can be concluded that the API that is being used in the pre-formulation studies and ultimately in the formulation process is pure.

i. Differential Scanning Calorimetry (DSC):

The Differential Scanning Calorimetry (DSC) of Paclitaxel showed an endothermic reaction at 221.5^{0}C.

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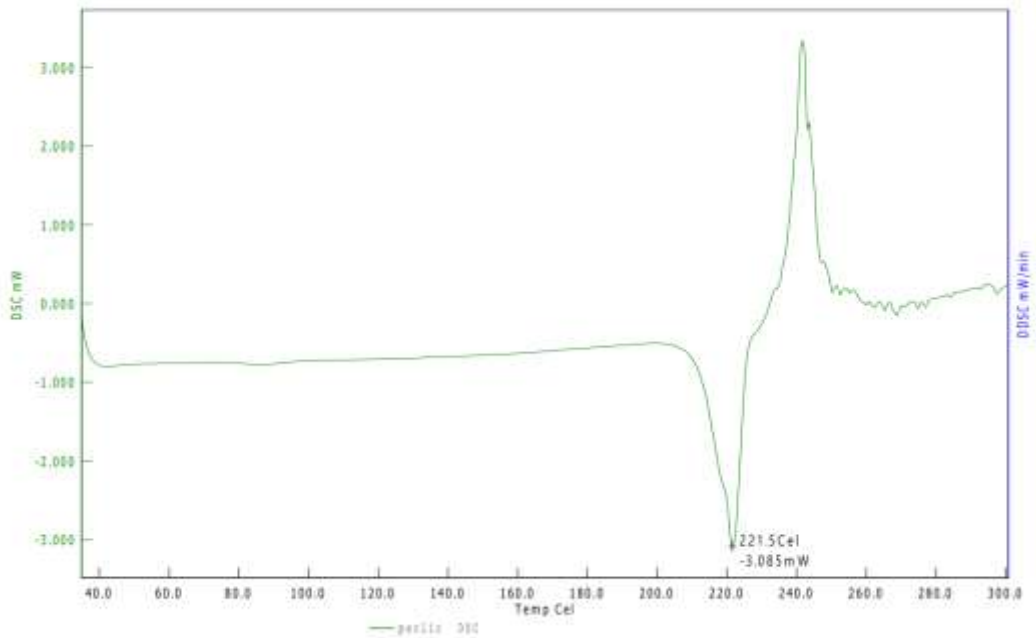
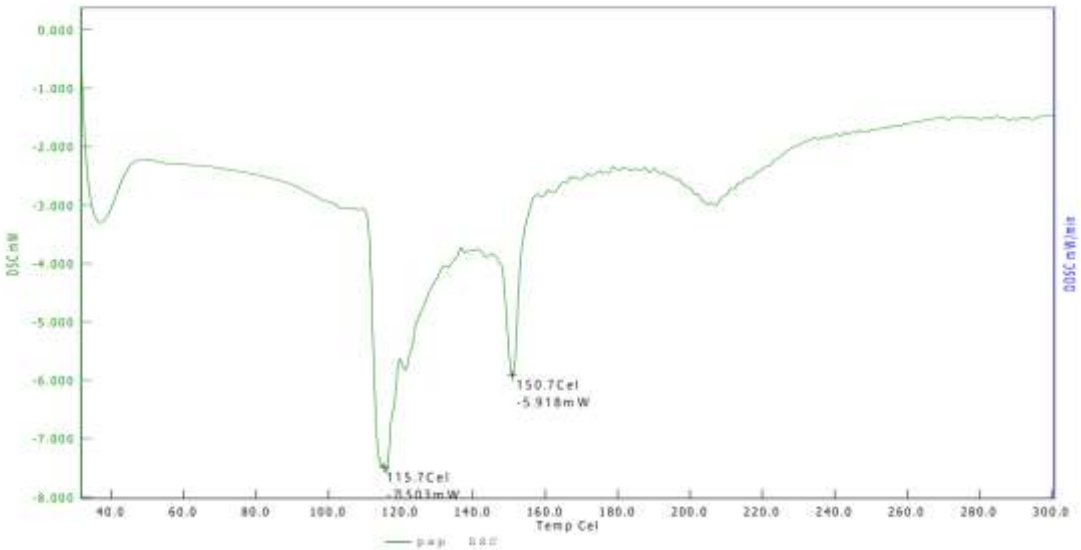


Differential Scanning Calorimetry (DSC) Of Paclitaxel

The Differential Scanning Calorimetry (DSC) of Papain showed an endothermic reaction at 115.7^{0}C and 150.7^{0}C.



Differential Scanning Calorimetry (DSC) Of Papain

Result: It can be concluded that the API and the herbal extract that is being used in the pre-formulation studies and ultimately in the formulation process is pure after comparing with the standard Differential Scanning Calorimetry (DSC) of Paclitaxel and Papain .

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3. RESULT FOR 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 as following show in Table.

Batch	Drug Content %	Entrapment efficiency %	Production yield %	pH	Drug Release %	Syringeability (Needle)
1	0	0	89	7.20	0	18 gauze
2	0	0	93	7.28	0	18 gauze
3	0	0	80	7.56	0	18 gauze
4	82	84	90	7.20	90	18 gauze
5	**87**	**89**	**95**	**7.44**	**98**	**18 gauze**
6	85	87	93	7.32	95	18 gauze
7	0	0	78	7.29	0	18 gauze
8	0	0	86	7.39	0	18 gauze
9	0	0	75	7.55	0	18 gauze
10	76	78	84	7.60	80	18 gauze
11	77	80	89	7.48	88	18 gauze
12	71	75	82	7.59	78	18 gauze

a. **Batch Optimization**:

The optimized batch was considered to be Batch 5 due to it's highest drug entrapment efficiency(89%), highest drug content(87%), highest production yield(95%), more reproducibility, high drug release(98%), pH(7.44) and syringeability (needle 18 gauze) in comparison with other designed batches.

b. **Drug release of the optimized batch (Batch 5):**

Time (mins)	% Cumulative Drug Release
0	0
30	28
60	42
90	52
120	65
150	81
180	91
210	98

Drug Release Of The Optimized Batch (Batch 5)

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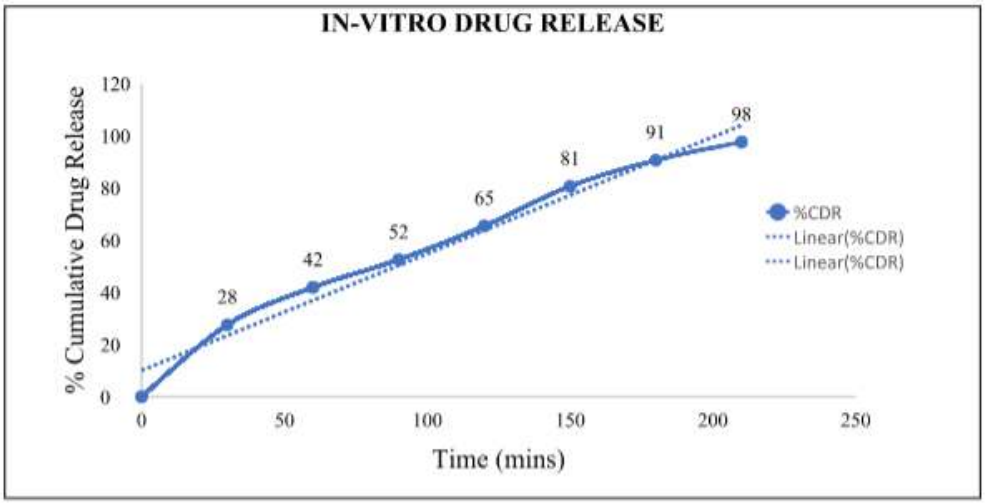
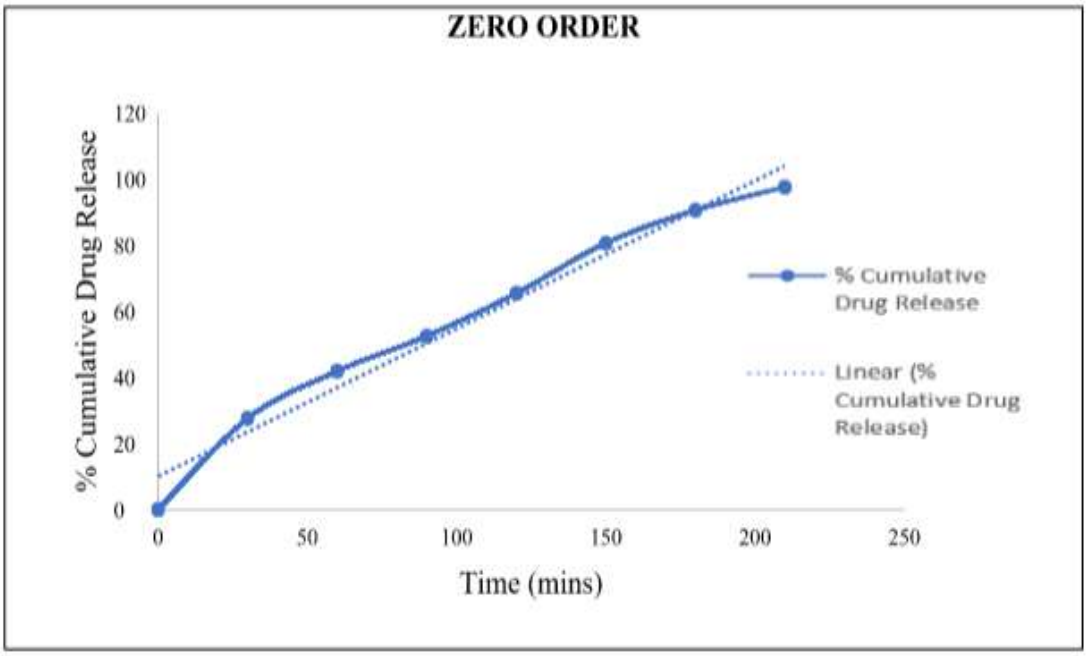


Drug Release Of The Optimized Batch (Batch 5)

c. Release kinetics studies:

The optimized batch(Batch 5) was subjected to release kinetics studies and the results are as follows:



Zero Order Plot Of The Optimized Batch (Batch 5)

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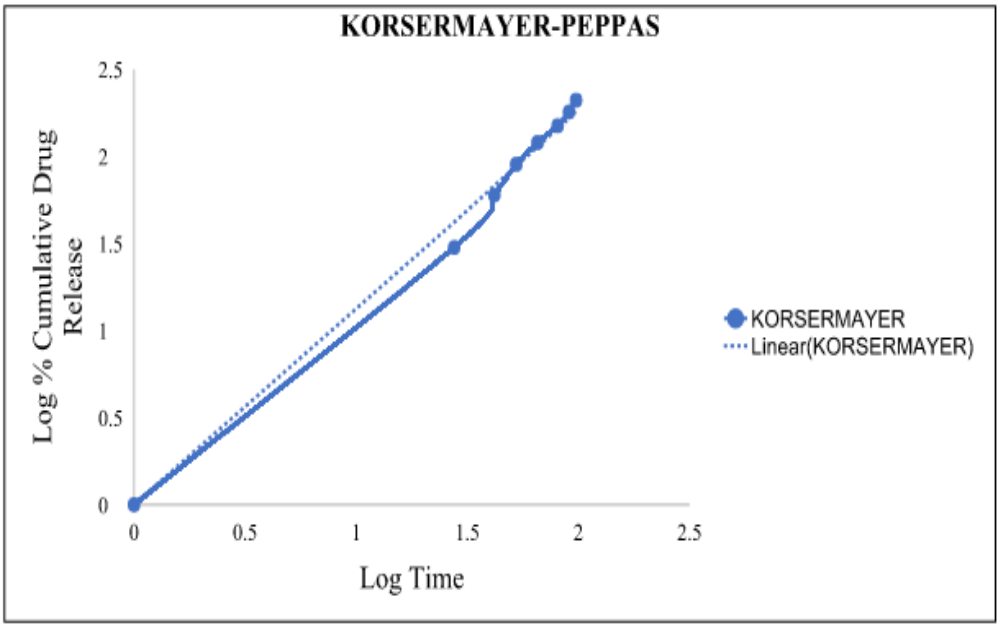
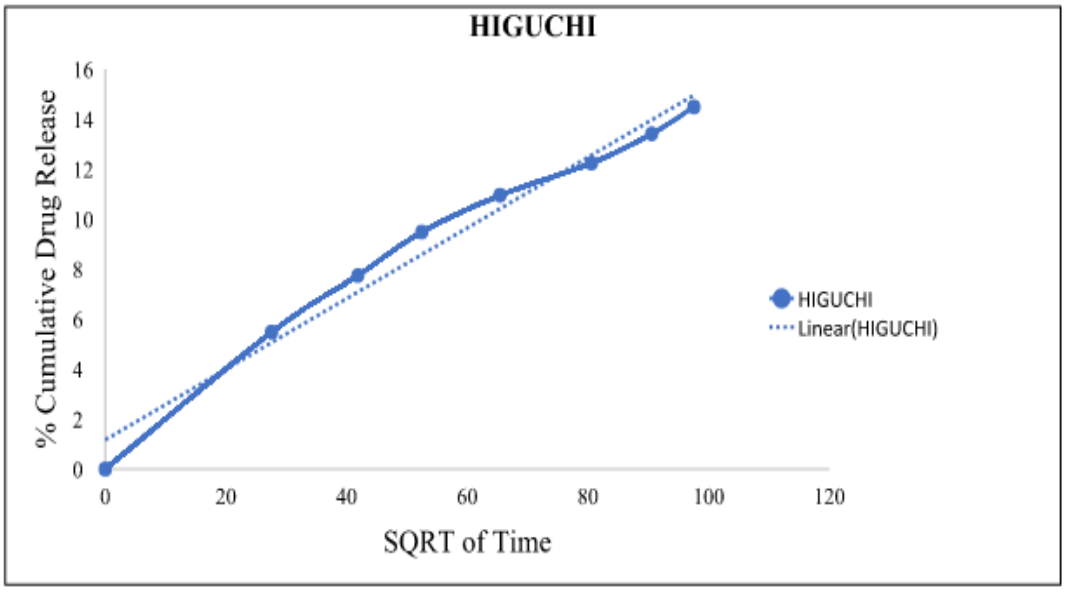


Korsermayer-Peppas Plot Of The Optimized Batch (Batch 5)



Higuchi Plot Of The Optimized Batch (Batch 5)

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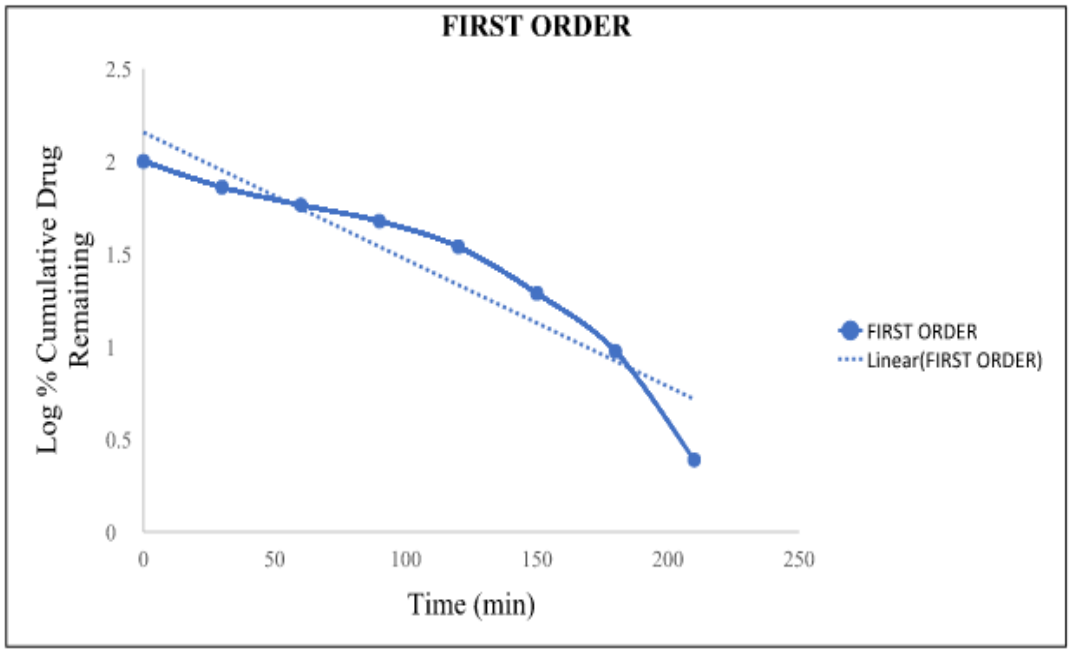


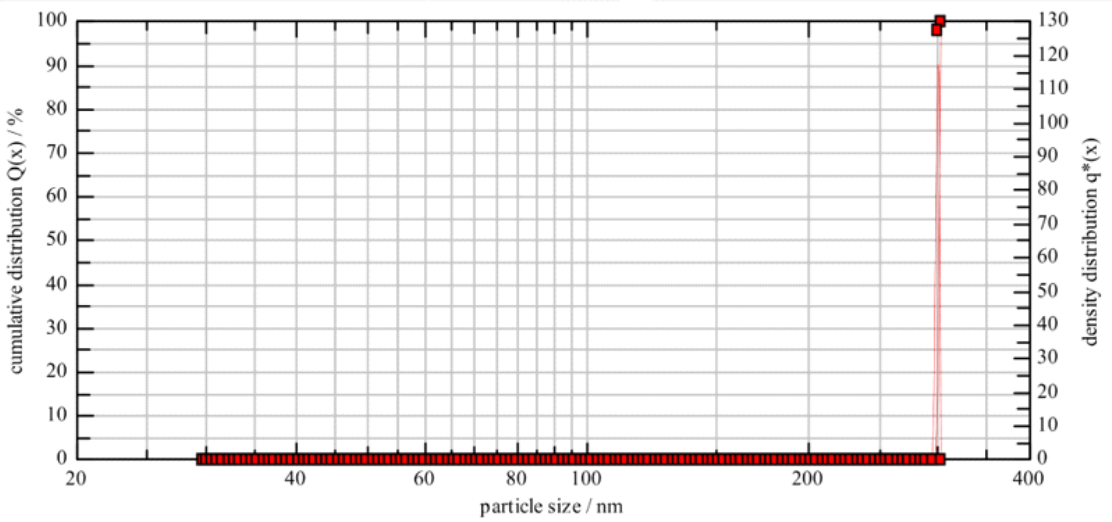
First Order Plot Of The Optimized Batch (Batch 5)

Release Kinetic Model	Regression Coefficient (R^2)
Zero Order	0.9738
Korsermayer-Peppas	0.9921
Higuchi	0.9768
First Order	0.9853

Kinetic Study R^2 Values Of The Optimized Batch (Batch 5)

d. Particle Size Analysis:

The particle size of the optimized formulation (Batch 5) was found to be 300.01 nm and poly dispersity index was found to be 503.94 %.



Particle size analysis Of The Optimized Batch (Batch 5)

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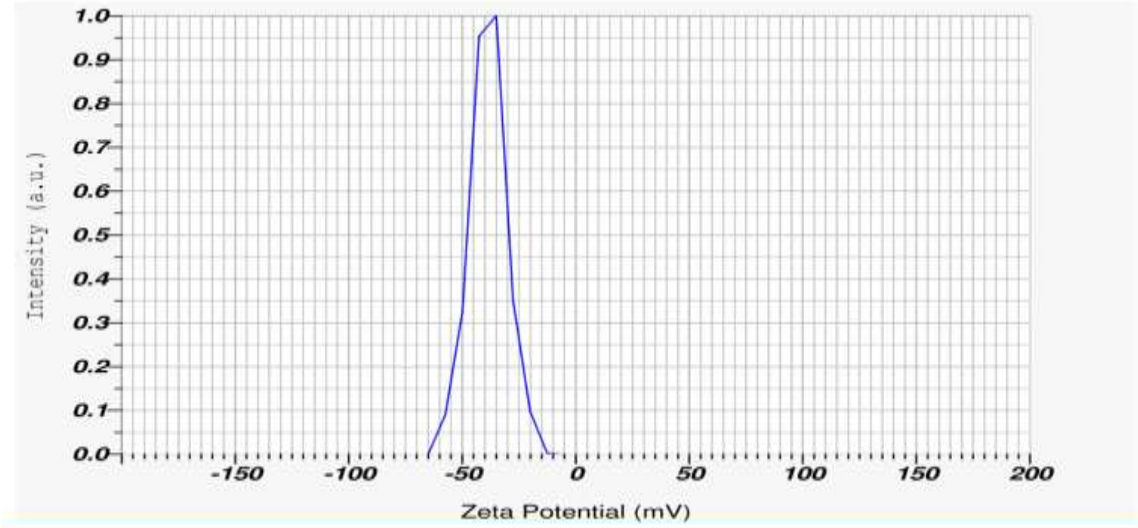
The cumulative distribution in the particle size of the optimized formulation (Batch 5) was found to be 100% at 302.73 x_0/nm.

x_0/nm	Q_3/%	x_0/nm	Q_3/%	x_0/nm	Q_3/%	x_0/nm	Q_3/%
30.27	0.00	54.08	0.00	96.60	0.00	172.57	0.00
30.83	0.00	55.07	0.00	98.37	0.00	175.73	0.00
31.39	0.00	56.08	0.00	100.17	0.00	178.94	0.00
31.97	0.00	57.10	0.00	102.00	0.00	182.22	0.00
32.55	0.00	58.15	0.00	103.87	0.00	185.55	0.00
33.15	0.00	59.21	0.00	105.77	0.00	188.94	0.00
33.75	0.00	60.29	0.00	107.71	0.00	192.40	0.00
34.37	0.00	61.40	0.00	109.68	0.00	195.92	0.00
35.00	0.00	62.52	0.00	111.68	0.00	199.51	0.00
35.64	0.00	63.66	0.00	113.73	0.00	203.16	0.00
36.29	0.00	64.83	0.00	115.81	0.00	206.87	0.00
36.95	0.00	66.01	0.00	117.93	0.00	210.66	0.00
37.63	0.00	67.22	0.00	120.08	0.00	214.51	0.00
38.32	0.00	68.45	0.00	122.28	0.00	218.44	0.00
39.02	0.00	69.70	0.00	124.52	0.00	222.43	0.00
39.73	0.00	70.98	0.00	126.80	0.00	226.50	0.00
40.46	0.00	72.28	0.00	129.12	0.00	230.65	0.00
41.20	0.00	73.60	0.00	131.48	0.00	234.87	0.00
41.96	0.00	74.95	0.00	133.88	0.00	239.16	0.00
42.72	0.00	76.32	0.00	136.33	0.00	243.54	0.00
43.50	0.00	77.72	0.00	138.83	0.00	248.00	0.00
44.30	0.00	79.14	0.00	141.37	0.00	252.53	0.00
45.11	0.00	80.59	0.00	143.95	0.00	257.15	0.00
45.94	0.00	82.06	0.00	146.59	0.00	261.86	0.00
46.78	0.00	83.56	0.00	149.27	0.00	266.65	0.00
47.63	0.00	85.09	0.00	152.00	0.00	271.53	0.00
48.50	0.00	86.65	0.00	154.78	0.00	276.50	0.00
49.39	0.00	88.23	0.00	157.61	0.00	281.55	0.00
50.30	0.00	89.85	0.00	160.50	0.00	286.71	0.00
51.22	0.00	91.49	0.00	163.43	0.00	291.95	0.00
52.15	0.00	93.16	0.00	166.42	0.00	297.29	0.00
53.11	0.00	94.87	0.00	169.47	0.00	302.73	100.00
product: PACLI				liquid: WATER			

Cumulative distribution in the particle size analysis Of The Optimized Batch (Batch 5)

e. Zeta Stability:

The zeta stability of particles in dispersion of the optimized formulation (Batch 5) was found to be -38.6 mV with electrophoretic Mobility -0.000299 cm2/Vs .



Zeta Potential Of The Optimized Batch (Batch 5)

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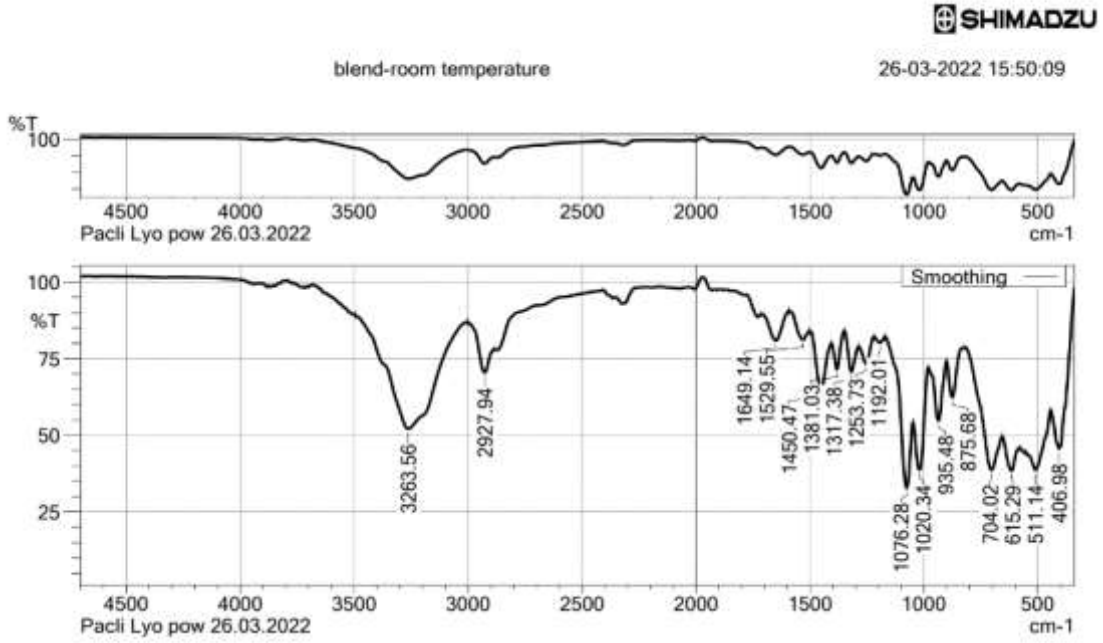
f. Fourier-Transform Infrared Spectroscopy (FTIR):

The interaction level of functional groups in the Fourier-Transform Infrared Spectroscopy (FTIR) is shown in following in Figure and Table.



Fourier-Transform Infrared Spectroscopy (FTIR) Of The Optimized Formulation (Batch 5)

Sr.no.	Peak Range cm^{-1}	Bond and Functional group
1	406.98, 511.14, 615.29, 704.02.	Benzene rings
2	875.68, 935.48.	C-O-C, C-OH, -NH Ether Alcohol Aliphatic amines
3	1020.34, 1076.28, 1192.01, 1253.73,	C-O-C, C-OH Ethers, Alcohols
4	1317.38, 1381.03, 1450.47, 1529.55	CH_3, CH_2 Alkanes, Alkenes
5	1649.14	-C=O Ester, Ketones, Aldehydes, Carboxylic acid
6	2927.94	-CH, $-CH_2$, $-CH_3$ Aliphatic groups
7	3263.56	-OH, -NH Alcohol, Amides, Amines

Fourier-Transform Infrared Spectroscopy (FTIR) Of The Optimized Formulation (Batch 5)

Result: The all ingredients of the optimized formulation (Batch 5) was found to be compatible with Paclitaxel after comparing with the standard Fourier-Transform Infrared Spectroscopy (FTIR) of the Paclitaxel, Papain and other ingredients.

g. Differential Scanning Calorimetry (DSC):

The Differential Scanning Calorimetry (DSC) of the optimized formulation (Batch 5) showed an endothermic reaction at 111.5^0C and 192.9^0C.

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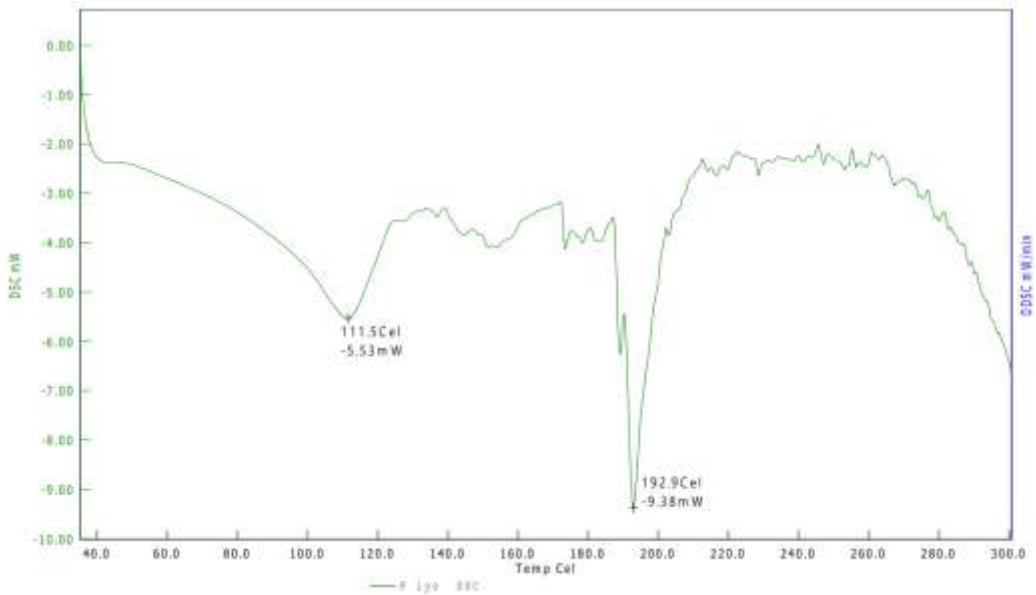
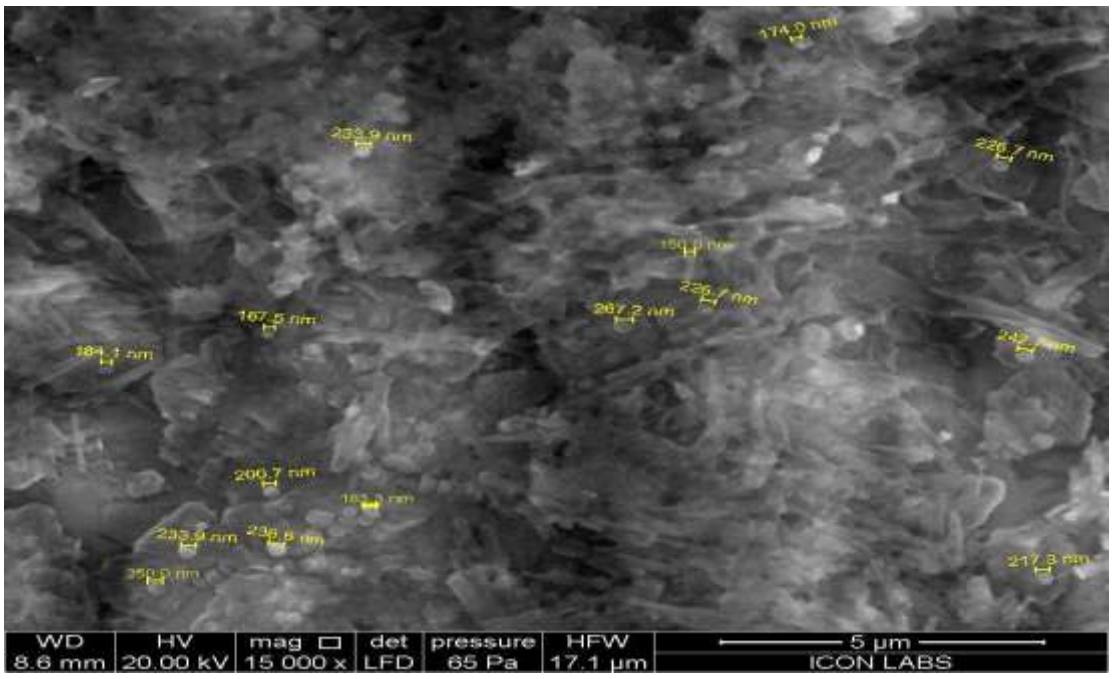


Differential Scanning Calorimetry (DSC) Of The Optimized Formulation (Batch 5)

Result: The all ingredients of the optimized formulation (Batch 5) was found to be compatible with Paclitaxel after comparison with the standard Differential Scanning Calorimetry (DSC) of Paclitaxel, Papain and other ingredients.

h. Particle Morphology:

The Scanning Electron Microscope (SEM) image of Paclitaxel loaded solid lipid nanoparticles shows that the nanoparticles in optimized batch 5 are spherical in shape and the particle size were found in the range of 150-267 nm.



Particle Morphology Of The Optimized Formulation (Batch 5)

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4. RESULT FOR STABILITY STUDIES:

The optimized formulation (Batch 5) was found to be stable for 3 months at 5±3◦ C and 25 ± 2◦ C/60 ± 5% RH. and the evaluation parameter are shown in following Table no.17

Evaluation Parameter	Initial	After 3 Months
Appearance	Off-White Powder	Off-White Powder
Drug Entrapment Efficiency (%)	89	88.6
Drug Content (%)	87	86.1
In Vitro Drug Release (%)	98	97.8

Stability Studies Of The Optimized Batch (Batch 5)

VIII. CONCLUSION:

As per literature survey the Paclitaxel-loaded solid lipid nanoparticles were prepared using Soy Lecithin and Oleic Acid as lipids and Papain as the herbal extract using various drug:lipid ratio by the technique of Solvent Emulsification and the prepared nano-emulsion was lyophilized using Freeze Drying technique. The drug: lipid ratio of Batch 5 with Paclitaxel (drug) 0.6 g, Soy Lecithin (lipid) 1.2g, and Oleic Acid (lipid) 0.6g gave satisfactory results and it was found to be more reproducible with highest drug content (%), drug entrapment efficiency (%) and in vitro drug release (%) so it was considered to be the optimized formulation. As per FTIR and DSC the Paclitaxel and Papain were found to be pure in nature. The maximum solubility of the Paclitaxel and Papain was found to be in methanol through pre-formulation studies. The optimized formulation was also found to be compatible with all the ingredients of the formulation as shown by FTIR and DSC results. The size of designed solid lipid nanoparticles was 300.01 nm. The optimized formulation (Batch 5) was found to be stable for 3 months at 5±3◦ C and 25 ± 2◦ C/60 ± 5% RH with no significant change in appearance, drug entrapment efficiency (%), drug content (%) and in vitro drug release (%).

REFERENCE:

[1]. Peer D, Karp JM, Hong S, Farokhzad OC, Margalit R, Langer R. Nanocarriers as an emerging platform for cancer therapy. Nano-Enabled Medical Applications. 2020 Nov 23:61-91.

[2]. Suri SS, Fenniri H, Singh B. Nanotechnology-based drug delivery systems. Journal of occupational medicine and toxicology. 2007 Dec;2(1):1-6.

[3]. Cho K, Wang XU, Nie S, Shin DM. Therapeutic nanoparticles for drug delivery in cancer. Clinical cancer research. 2008 Mar 1;14(5):1310-6.

[4]. Wilczewska AZ, Niemirowicz K, Markiewicz KH, Car H. Nanoparticles as drug delivery systems. Pharmacological reports. 2012 Sep 1;64(5):1020-37.

[5]. Bhatia S. Nanoparticles types, classification, characterization, fabrication methods and drug delivery applications. InNatural polymer drug delivery systems 2016 (pp. 33-93). Springer, Cham.

[6]. Hasan S. A review on nanoparticles: their synthesis and types. Res. J. Recent Sci. 2015;2277:2502.

[7]. Buabeid MA, Arafa ES, Murtaza G. Emerging prospects for nanoparticle-enabled cancer immunotherapy. Journal of Immunology Research. 2020 Jan 3;2020.

[8]. Lingayat VJ, Zarekar NS, Shendge RS. Solid lipid nanoparticles: a review. Nanoscience and Nanotechnology Research. 2017 Apr;4(2):67-72.

[9]. Awasthi R, Roseblade A, Hansbro PM, Rathbone MJ, Dua K, Bebawy M. Nanoparticles in cancer treatment: opportunities and obstacles. Current drug targets. 2018 Oct 1;19(14):1696-709.

[10]. Ekambaram P, Sathali Aa, Priyanka K. Solid Lipid Nanoparticles: A Review.

[11]. Priyadarshini K, Keerthi AU. Paclitaxel against cancer: a short review. Med chem. 2012 Nov;2(7):139-41.
[12]. Jones TM. Preformulation studies.
[13]. Savjani KT, Gajjar AK, Savjani JK. Drug solubility: importance and enhancement techniques. International Scholarly Research Notices. 2012;2012.
[14]. 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.
[15]. 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.
[16]. Devi TR, Gayathri S. FTIR and FT-Raman spectral analysis of Paclitaxel drugs. Int J Pharm Sci Rev Res. 2010;2(2):106-10.
[17]. 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.
[18]. 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.
[19]. 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.
[20]. 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.
[21]. 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.
[22]. 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.
[23]. Venishetty VK, Parikh N, Sistla R, Ahmed FJ, Diwan PV. Application of validated RP-HPLC method for simultaneous determination of docetaxel and ketoconazole in solid lipid nanoparticles. Journal of chromatographic science. 2011 Feb 1;49(2):136-41.
[24]. 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.
[25]. Devi TR, Gayathri S. FTIR and FT-Raman spectral analysis of Paclitaxel drugs. Int J Pharm Sci Rev Res. 2010;2(2):106-10.
[26]. Raza K, Singh B, Singal P, Wadhwa S, Katare OP. Systematically optimized biocompatible isotretinoin-loaded solid lipid nanoparticles (SLNs) for topical treatment of acne. Colloids and Surfaces B: Biointerfaces. 2013 May 1;105:67-74.