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Preparation, Evaluation And Optimization Of Lenvatinib As A Liposomes For Carcinoma

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ABSTRACT: Lenvatinib, a multi-targeted tyrosine kinase inhibitor, has shown promising anticancer activity against various malignancies. However, its clinical application is hindered by poor aqueous solubility, low bioavailability, and off-target toxicity. To address these limitations, liposomal drug delivery systems have emerged as an effective strategy to improve the pharmacokinetic and pharmacodynamic profiles of anticancer agents. In this study, we developed and characterized a novel liposomal formulation encapsulating Lenvatinib, aiming to enhance its therapeutic efficacy while minimizing adverse effects. The liposomes were prepared using a thin-film hydration method and optimized for particle size, encapsulation efficiency, and stability. Physicochemical characterization demonstrated uniform size distribution, high drug loading, and sustained release kinetics. In vitro studies revealed enhanced cellular uptake and cytotoxicity of Lenvatinib-loaded liposomes compared to free drug in various cancer cell lines. Furthermore, in vivo pharmacokinetic and biodistribution studies demonstrated prolonged circulation time and increased accumulation of Lenvatinib in tumor tissues following liposomal administration. Moreover, the liposomal formulation exhibited superior antitumor efficacy and reduced systemic toxicity in xenograft mouse models compared to the free drug. Overall, our findings suggest that the developed Lenvatinib-loaded liposomal formulation holds great promise as a targeted and efficacious anticancer therapy, warranting further preclinical and clinical investigations. This study highlights the potential of Lenvatinib-loaded liposomes as a promising approach for the targeted delivery of Lenvatinib, offering enhanced therapeutic efficacy and improved safety profile in the treatment of solid tumors.

KEY WORDS*:* *Lenvatinib, Liposomes. Anticancer, Drug delivery, Tyrosine kinase inhibitor, Formulation, Encapsulation, Nanomedicine, Targeted therapy.*

1. INTRODUCTION:

Cancer is a leading cause of death worldwide. In 2018, cancer statistics in the United States predicted more than 1.7 million new cancer cases and over 600,000 cancer-related deaths [1]. Various treatment strategies are available to help the patients and manage the disease, depending on the type and stage of the disease at diagnosis. This includes surgery to remove the tumour bulk, cytotoxic chemotherapy and radiotherapy to selectively kill the rapidly dividing and partially impaired cancer cells, targeted therapies directed towards specific genetic drivers of cancer, and immunotherapy to stimulate the innate and acquired immune system against malignant cells. The number of cancer survivors has increased in recent decades, partly due to advances in early detection, but also because of the improved treatment outcomes from new therapeutic strategies. However, despite this large repertoire of treatments, cancer cells develop resistances to therapies and disseminate from the primary tumour to distant sites forming metastases [2] which ultimately kill the patient. New treatments, consisting of novel combinations of existing therapies and new innovative therapeutics, are urgently needed, particularly in the case of metastatic disease.

Tumours have been historically perceived as groups of cells with deregulated growth that proliferate without control and, at later stages, metastasise. However, tumours are not exclusively cells behaving independently and are, instead, complex structures of malignant cells that constantly interact with the surrounding microenvironment and change because of accumulating mutations. The microenvironment is a key factor during cancer development and often has tumour-promoting functions. The main components of the tumour microenvironment (TME) are non-malignant cells that secrete cytokines, chemokines, growth factors, inflammatory and matrix remodeling enzymes to build the modified tumour stroma, as well as blood and lymphatic vasculature. These non-malignant cells have also a profound effect on the efficacy of anticancer therapies, and include cancer-associated fibroblasts, vascular endothelial cells, and cells of the immune system, such as tumour-infiltrating lymphocytes, tumour-associated macrophages, and myeloid-derived suppressor cells. Common non-cellular features of the TME are hypoxia, nutrient deprivation, low pH, and high interstitial fluid pressure [3].

Drug candidates have been developed to target the components of the TME in order to overcome acquired resistances, prevent metastasis of cancer cells, and improve therapeutic efficacy. However, many of these compounds are of hydrophobic nature, resulting in poor aqueous solubility and may be rapidly eliminated, poorly adsorbed if given orally, and/or may present undesired biodistribution. Liposomes are a well-described drug delivery system that has transitioned to clinical applications with proven capabilities that can overcome these problems [4]. Liposomes are spherical lipid vesicles, typically with a mean diameter of 100 nm and composed of a phospholipid bilayer with or without cholesterol. They have an aqueous core, and the bilayer itself creates a hydrophobic region. In addition to the encapsulation of hydrophobic drugs, extension of blood circulation time, and increase in drug exposure to the tumour tissue, liposomes also facilitate the distribution of the associated drug to the TME. Although heterogeneous, passive accumulation of liposomal formulations occurs through the enhanced permeability and retention (EPR) effect, a phenomenon that is based on the prolonged circulation of liposomes, the leaky vasculature surrounding the tumour that allows selective extravasation of liposomes, and the impaired tumour-associated lymphatic system, that prevents the elimination of vesicles from the tumour tissue [5]

There is a great potential for liposomal formulations to enhance the delivery of compounds with potential anticancer activity—compounds synthesized to modulate the TME and reactivate the tumour-associated immune response. Lenvatinib (Len) is an oral multi-tyrosine kinase inhibitor (MKI) that identifies PDGF receptor α, FGF receptors 1–4, VEGF receptors 1–3, c-KIT and RET. Len has been approved as a first-line oral systemic treatment for unresectable HCC by the U.S. Food and Drug Administration However, Len still causes severe gastrointestinal and vascular adverse reactions in clinical settings. Therefore, it is beneficial for patients to develop a novel drug delivery system for Len that has lower adverse reactions and higher efficiency. [6]

In this investigation, we prepared an injectable micelle-based anti-tumor drug delivery system that uses HA-modified Len-loaded micelles to decrease adverse reactions and increase drug delivery efficiency specifically against CD44 protein for treating HCC. Initially, Len was selected as a model drug for anti-HCC first-line treatment in clinical settings. To further improve the tumor-targeting of Len, we synthesized a HA-PG10-C18 derivative that can specifically attach to CD44 protein on the membranes of H22 cells. The HA-PG10-C18 derivative was then self-assembled with Len to form HA@Len-M using a lyophilization-hydration method. We also characterized the detailed physicochemical properties and stability of HA@Len-M. Subsequently, *in vitro* experiments were performed using H22 cells to evaluate the ability of the micelles to deliver payloads and suppress development of tumor cells.[6]

1.1 Study Design and Treatment:

Patients in this single-arm, open-label multicenter study of lenvatinib monotherapy for advanced HCC (NCT00946153) received daily oral administration of 12 mg lenvatinib (12-mg QD) in 28-day cycles until disease progression, unacceptable toxicity, or withdrawal of con sent. Dose interruption and sequential reduction of lenvatinib (to 8- and 4-mg QD) were permitted for drug-related adverse events. Once reduced, the dose could not be re-escalated. The study drug was discontinued if patient recovery time was > 2 weeks.

1.2 Introduction of Lenvatinib:

Lenvatinib is a multikinase inhibitor primarily used in the treatment of certain types of cancer. It works by inhibiting the activity of specific enzymes that promote the growth and spread of cancer cells.

1.3 Dosage and Administration:

The dosage of lenvatinib varies based on the type of cancer being treated and the patient's body weight. It is administered orally in capsule form. For instance:

For thyroid cancer: A common dose is 24 mg once daily.

- For hepatocellular carcinoma: The dose is typically 8 mg or 12 mg once daily based on body weight (8 mg for patients <60 kg and 12 mg for patients ≥60 kg).
- For renal cell carcinoma and endometrial carcinoma: The dose may be adjusted when used in combination with other therapies.

1.4 Mechanism of Action:

Lenvatinib targets multiple receptor tyrosine kinases (RTKs) involved in tumor growth and angiogenesis. These include:

1.4.1. Vascular Endothelial Growth Factor Receptors (VEGFR) 1-3: These are critical for angiogenesis, the process of forming new blood vessels, which tumors need to grow and metastasize.

1.4.2. Fibroblast Growth Factor Receptors (FGFR) 1-4: These play a role in cell proliferation, differentiation, and angiogenesis.

1.4.3. Platelet-Derived Growth Factor Receptor Alpha (PDGFRα): Involved in the growth and development of blood vessels.

1.4.4. KIT: A receptor involved in cell survival and proliferation.

1.4.5. RET: A receptor associated with cell growth and differentiation.

By inhibiting these pathways, lenvatinib reduces tumor vascularity and induces apoptosis (programmed cell death) in cancer cells.

1.5. Advantages Of Liposome Drug Delivery Systems in Cancer Treatment:

Liposome drug delivery systems have shown significant promise in cancer treatment due to their unique properties and capabilities. Here are the key advantages:

1.5.1. Enhanced Permeability and Retention (EPR) Effect:

- **Passive Targeting**: Tumors often have leaky vasculature, which allows liposomes to accumulate more in tumor tissues compared to normal tissues. This passive targeting helps to concentrate the drug in the tumor site, minimizing exposure to healthy cells and reducing side effects.
- **Active Targeting**: **Functionalization**: Liposomes can be modified with ligands, antibodies, or other targeting moieties that bind specifically to receptors on cancer cells, enhancing selective drug delivery to the tumor and sparing normal tissues.

1.5.2. Improved Pharmacokinetics and Pharmacodynamics:

- **Prolonged Circulation Time**: Liposomes can evade the immune system and stay longer in the bloodstream, allowing more time for the drug to reach and penetrate the tumor.
- **Controlled Release**: Encapsulated drugs can be released in a controlled manner, ensuring a sustained therapeutic effect and maintaining drug levels within the therapeutic window for longer periods.

1.5.3. Enhanced Solubility and Stability:

- **Encapsulation of Hydrophobic Drugs**: Many anticancer drugs are hydrophobic and have poor solubility in water. Liposomes can encapsulate these drugs, improving their solubility and bioavailability.
- **Protection from Degradation**: Liposomes protect encapsulated drugs from degradation by enzymes and other factors in the bloodstream, enhancing drug stability and effectiveness.

1.5.4. Multifunctionality:

- **Co-delivery of Multiple Agents**: Liposomes can carry multiple therapeutic agents simultaneously, such as chemotherapeutic drugs and gene therapies, allowing for combination treatments that can be more effective against cancer.

1.5.5. Biocompatibility and Reduced Immunogenicity:

- **Natural Composition**: Liposomes are typically made from phospholipids, which are naturally occurring in the body, making them biocompatible and reducing the risk of adverse immune reactions.

• **PEGylation**: Coating liposomes with polyethylene glycol (PEG) further reduces immunogenicity and extends their circulation time by preventing recognition and clearance by the immune system.

1.5.6. Customized Drug Release Profiles:

• **pH-Sensitive Liposomes**: Liposomes can be engineered to release their payload in response to the acidic environment of tumors, providing targeted drug release within the tumor microenvironment.

• **Temperature-Sensitive Liposomes**: These can be designed to release drugs in response to hyperthermia, which is sometimes used in conjunction with cancer treatments.

2.0 MATERIALS:

Lenvatinib, soya lecithin, Cholestrol, Ethanol, Calcium chloride these are the materials used in formulation of Lenvatinib loaded Liposomes.

3.0 EXPERIMENTAL WORK:

3.1 Preformulation study:

Preformulation study is defined as the study of the physical and chemical properties of the drug molecular prior to the compounding process. It is the most important study in the development of any new dosage form of a drug. The main objective of the pre formulation studies are as follows:

• To establish the physicochemical parameter of new drug activity.

• To establish its compatibility with common excipients.[12]

3.1.1 Organoleptic Properties:

The physical condition, color, and odor of the Lenvatinib sample were assessed visually.

3.2 Melting Point Determination:

The melting point is one of the key parameters used to identify a drug and its crystalline state. Lenvatinib melting point was determined using the open capillary tube method. To close a capillary tube, heat one end in a flame for 2-3 minutes and rotate it continuously. Take Lenvatinib as fine powder. Dip the capillary tube's open end in finely powdered Lenvatinib. Tap the capillary tube gently on the table to fill it with the compound up to 1-2 cm. Dip the thermometer into a Thieles tube containing paraffin oil after attaching the capillary with a rubber band. Keep a constant watch on the temperature and record it as soon as possible.[13]

3.3 Fourier Transforms Infrared Analysis:

Fourier Transform infrared spectroscopy was used to characterise the likely structural alteration generated in the atorvastatin sample (Agilent Technologies, carry 630 FTIR). The sample was analyzed at 4000 to 400 cm-1. The solid drug was kept on the sample-holding surface for examination. In a similar manner, polymer spectra were obtained.

3.4 Drug Excipients Compatibility Study:

The drug excipient compatibility study was conducted using Fourier Transform infrared spectroscopy (FTIR spectrophotometer, Agilent Technologies, carry 630 FTIR). The drug excipient compatibility study was conducted using Fourier Transform infrared spectroscopy (FT-IR spectrophotometer, Agilent Technologies, carry 630 FTIR) with a sample analysis range of 4000 to 400 cm. The sample's analysis range was 4000–400 cm-1. The procedure entails applying a well-mixed mixture of drug and polymer (1:1) to a surface that will hold samples for examination. The first range of the medication and polymer mixture was taken, and the mixture was then stored in a stability chamber for a month at 40°C and 75°R. The sample mixture was examined after one month using Fourier Transform infrared spectroscopy (FT-IR spectrophotometer, Agilent Technologies, carry 630 FTIR). The drug and excipient compatibility study was carried out by comparing the mixed sample's initial and one-month-after spectra to determine whether the drugs and excipients were compatible. [14]

3.5.5 Calibration Curve of lenvatinib in methanol:

Preparation of lenvatinib stock and working solutions

To obtain a concentration of 100µg/ml (stock I), 10 mg of lentanib was dissolved in methanol and the volume was raised to 100 ml using methanol. 0.2 ml of stock I (100µg/ml) was taken to create different concentrations ranging from 2 to 12µg/ml. Take 0.4 ml, 0.6 ml, 0.8 ml, 1.0 ml, and 1.2 ml and dilute with the same dilution solution up to 10 ml to create the calibration curve. Absorbance was calculated at 254 nm per milliliter versus

absorbance using a UV Spectrophotometer. A linear regression analysis was done on absorbance data points. A straight-line equation (YmxC) was constructed in order to facilitate the calculation of the dosage.[15]

Absorbance Slope x concentration + intercept

3.6 METHOD OF PREPARATION:

Preparation of lenvatinib loaded liposomes by thin film hydration method:

Through the use of the thin film hydration approach, multi-lamellar vesicles (MLV) liposomes were created. The lipid phase of these liposomes was composed of mixes of cholesterol and phosphatidyl choline in various molar ratios (see table). In short, the lipid combination and 50 mg of lenvatinib were dissolved in a 3:3 v/v chloroform: methanol mixture, which was then extracted under vacuum at 45°C. This process produced a thin layer of dry lipid on the flask wall, which was then made using a rotary flash evaporator. The film was hydrated by adding phosphate buffer (pH 7.4) and vigorously shaking it with a vortex mixture until vesicles formed, following the full elimination of all solvent remnants. Using a Sonicator, the liposomes were further decreased in size to create tiny unilamellar vesicles. [11]

Table.no.3: Batch made on basis of different concentration of drug:

Formulation code	Ratio of CL:CH	Amount of Soya Lecithin (mg)	Amount of Cholesterol (mg)
L1	8:0	200	0
L2	7:1	175	25
L3	6:2	150	50
L4	5:3	125	75
L5	4:4	100	100
L6	3:5	75	125
L7	2:6	50	150
L8	1:7	25	175

3.7 OPTIMIZATION OF LIPOSOME PREPARATION:

Since the final outcome of the liposomal preparation is determined by the coating, a thin, homogenous coating is desirable. The rotational speed was varied between 60 and 100 rpm during the hydration and film-generating processes.

3.7.1 Speed of the rotary evaporator:

The flask was rotated in rotary flash evaporator at 80 rpm for 30 minutes in thermostatically controlled water bath at 40°C under vacuum 900 mm Hg. The organic solvent was slowly removed by this process such that a very thin film of dry lipids was formed on the inner surface of the flask.

3.7.2 The ratio and volume of solvent system:

The solvent system was modified by mixing varying volumes of two organic solvents, methanol and chloroform. The film's homogeneity was evaluated and it was tested in 3:1, 3:2, and 3:3 ratios.

3.7.3 pH of the hydrating media:

It was looked into how the pH of the phosphate buffer affected the formulation. pH affects the drug's trapping in the liposomes. After adjusting the hydration buffer's pH to values that were closer to the drug's pka, entrapment efficiency was determined. Distilled water and phosphate buffer with pH values of 5.2, 6.8, and

7.4 were used as hydrating medium. We looked at each formulation's trapping efficiency. [16]

3.8 EVALUATION OF LIPOSOMES:

3.8.1 Physical Appearance:

Visual inspection was used to assess the organoleptic qualities of the Liposomes preparations, including color, odor, and physical appearance

3.8.2 Determination of practical yield: The following formula was used to compute the production yield after the solid excipients and drug were precisely weighed before the preparation and after the formulation and drying of the Liposomes in the lyophilizer.

$$\mathbf{PY} = \frac{\textbf{Amount of product obtained}}{\textbf{Total amount of solid used in the preperation of drug (drug+excipient)}} \mathbf{X\ 100}$$

3.8.3 Determination of vesicle entrapment efficiency:

A clear supernatant was generated by centrifuging the liposomal pallet, or drug-loaded Liposomes, at 6000 rpm for 30 to 45 minutes using a mini spin centrifuge, in order to separate it from the aqueous phase. Using a direct method, the amount of drug loaded was estimated. [34]

Direct Method:

Decanting the supernatant followed centrifugation. The pallet that was created underwent phosphate buffer washing and vertexing to eliminate any free medication that had become adsorbed on the surface of the Liposomes. To totally separate the unentrapped medication, this process was carried out three times. After that, the pallet was dissolved in methanol and subjected to a 20-minute ultrasound (which caused the pallet to rupture and released the medication that had been trapped in the solution). Using the devised analytical approach, UV spectrophotometry was used to examine the resultant methanolic solution.

$$\textbf{\% Entrapment Efficiency (E.E)} \frac{\textbf{Amount of drug quantified in the pellet}}{\textbf{Total amount of drug added}} \mathbf{X\ 100}$$

3.8.4 Fourier Transform Infrared Spectroscopy:

The drug's identity and the way it interacts with polymers are both determined by the FTIR spectra. FTIR (Bruker Alpha-T. India) equipment was used to obtain the FTIR spectra of a batch of optimal solid dispersion preparations and pure medication. The process involved spreading a sample in excess of potassium bromide almost exactly at a 1:100 ratio thoroughly mixing the liquid and then storing it in a sample holder for examination. A spectrum containing potassium bromide (1:100) and pure medicine was obtained. The batch of solid dispersion with potassium bromide (1:100) that was spectrum optimized was taken. Over the wave number range of 4000-400 cm-1, the spectrum was examined.

3.8.5 Determination of particle size distribution and zeta potential:

Using the laser diffraction method, the size of the lenvatinib-loaded Liposomes was ascertained (Malvern 2000 SM: Malvern Instruments, Malvern, UK). The 908-scattering angle was used to measure the particle sizes. The average particle size was calculated and reported in terms of d (0.9) mm after the samples were distributed in distilled water. At a temperature of 25°C, the zeta potential was determined using the laser Doppler electrophoretic mobility measurements with the Zeta sizer 3000 (Malvern Instruments). [35]

3.8.6 Determination of Solubility in Distilled Water:

Solubility of pure and all batches of Liposomes in distilled water, which is produced by mixing an excess of the drug in a conical flask with 10 milliliters of distilled water. To guarantee saturation, this conical flask was placed on an orbital shaker for a full day. Clear supernatant was filtered after equilibrium solubility was reached, and the resulting sample was then subjected to the proper dilutions before being examined using a UV spectrophotometer set to 254 nm. 153). [36-37].

3.8.7 Differential Scanning Calorimeter Analysis:

Thermal properties of pure drug and optimized batch of Liposomes was analyzed by DSC (TA Instruments, USA, Model: SDT 2960) to optimized batch. Indium standard was used to calibrate the DSC temperature and enthalpy scale. Nitrogen was used as the purge gas through DSC cell at flow rate of 50 ml per min and 100 ml per min through the cooling unit. The sample (5-10mg) was heated in a hermetically sealed aluminum pans. Heat runs for each sample were set from 0 to 300°C at a heating rate of 10°C/ min [49].

3.8.8 In-Vitro Drug Release Studies:

Using the spinning paddle method of the USP II device, a dissolution test was conducted. A 100-rpm stirring rate was used. Phosphate buffer with a pH of 6.8 served as the dissolving media. The sample was obtained using a dialysis bag, and following the test, the dissolving medium was kept at 900 ml by adding 5 ml of sample at regular intervals. The sample was then removed, filtered, and replaced with 5 ml of new dissolution medium to maintain the sink condition. Dilutions were made as needed. Proceed with a spectrophotometric analysis at 254 nm. For every sample, the dissolving experiments were run in triplicate. It directs product development and formulation toward the optimization of products. [38]

Table no.4. Parameters for dissolution profile:

Sr.no	Parameter	Specifications
01	Dissolution medium	900 ml PH 6.8
02	Temperature	37^0C
03	Rotation speed	100 RPM
04	Sample volume withdrawn	5 ml
05	Time interval	45 min.

4.0 RESULT AND DISCUSSION:

4.1 Preformulation study:

4.1.1 Organoleptic properties:

Table no.5: Result of organoleptic properties of Lenvatinib:

Sr.no.	Parameters	Observations
1	Physical state	Solid crystalline
2	Colour	Pale yellow
3	Odor	Odorless or with a faint odor

4.1.2 Melting Point Determination:

Table no. 6. Melting point:

Drug	Melting point	Observed Melting point
Lenvatinib	**>216^{0}C**	**219^{0}C**

4.1.3 Fourier Transforms Infrared Analysis:

Lenvatinib Drug:

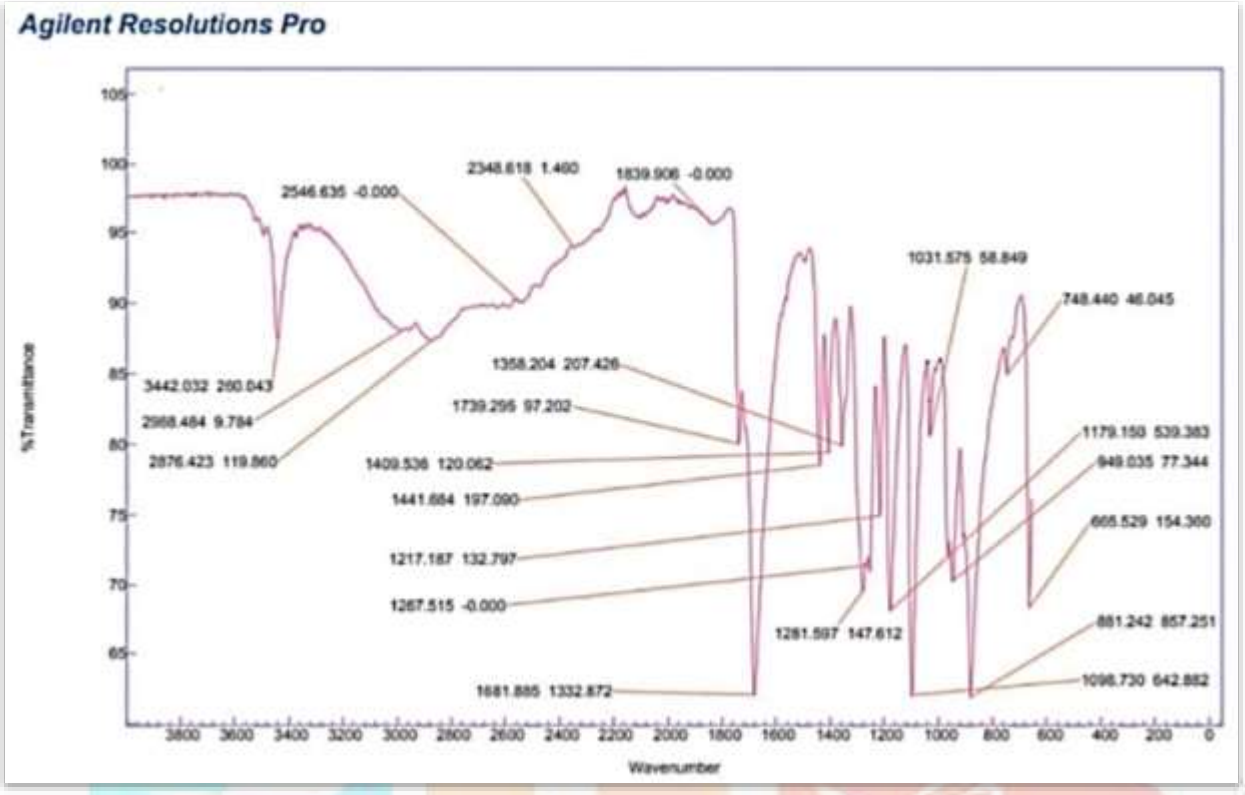


Fig. FTIR Spectra of Lenvatinib Drug

Table No.7: Major Observed IR Peaks of Lenvatinib:

Sr. No.	Functional groups	Peak Position (cm^{-1})	Indication
1.	Alkane halide	653	C - H Bending
2.	Benzene Ring	857	CH3 Bending
3.	Amine	1180	C =C Stretching
4.	Carboxylic acid	1321	C-O Stretching
5.	alkane	1442	C-H Stretching

4.1.4 FTIR of Drug and Excipient Mixture:

Drug excipients compatibility study was done by using FTIR Spectra, from this graph it was proved that there is no any changes in the IR spectra of physical mixture of drug and excipients.

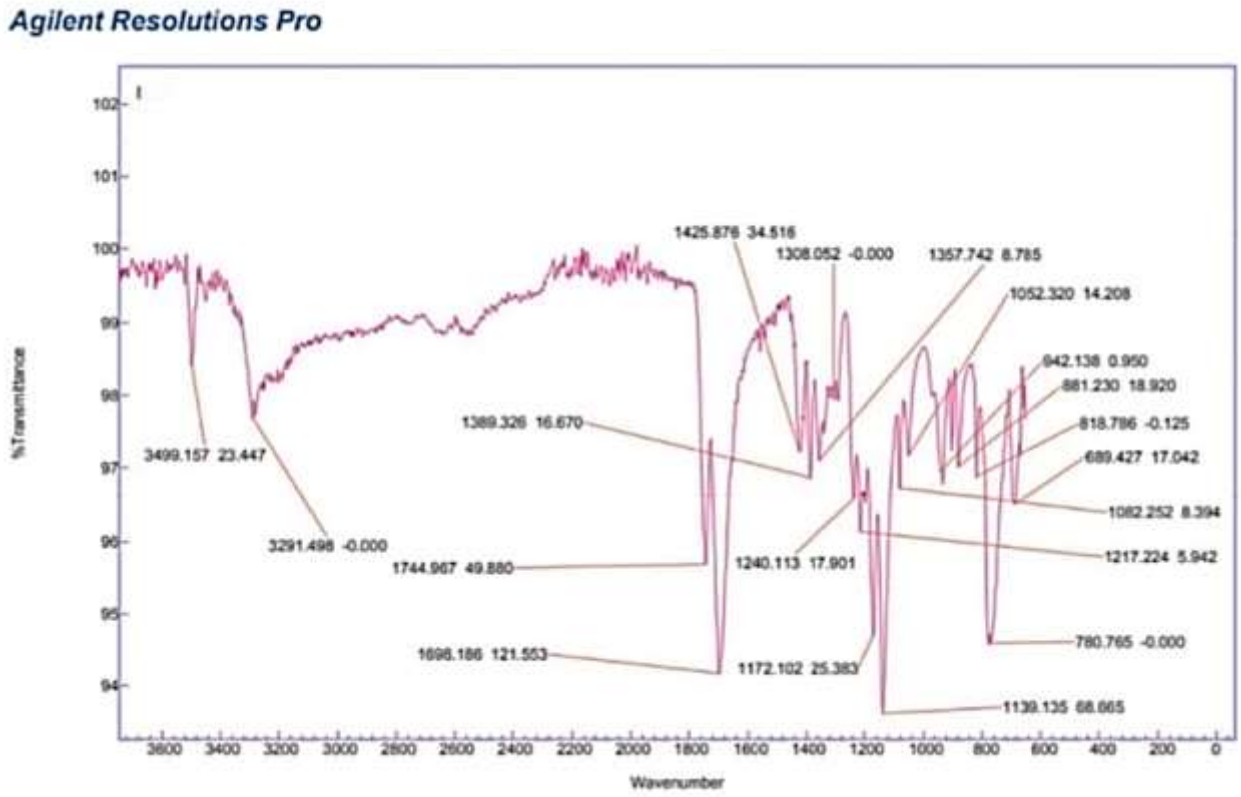


Fig. FTIR Spectra of Lenvatinib Drug and Excipient mixture

Fig: Calibration curve of Lenvatinib in phosphate buffer pH 6.8

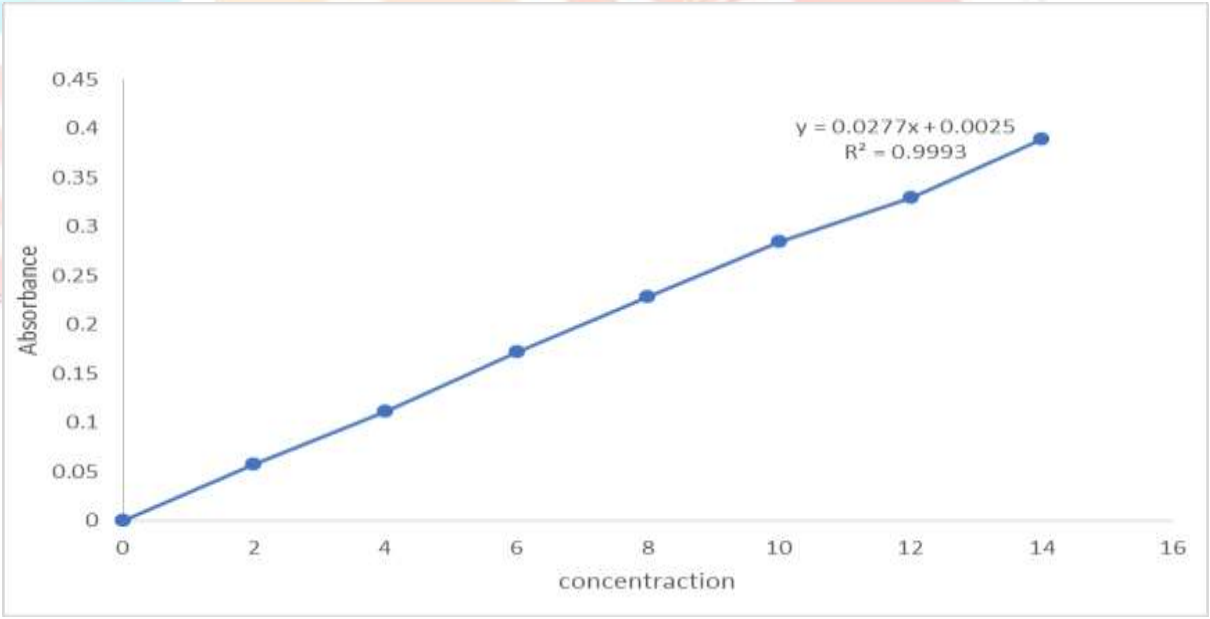


Table No.: Various Constant for Calibration Curve in phosphate bufferpH 6.8

Parameter	Slope	Intercept	R ^ 2
Value for calibration curve in phosphate buffer pH 6.8	0.027	+ 0.0025	0.9993

4.1.5. Calibration Curve of Lenvatinib in methanol:

The graph of concentration vs. Absorbance for pure Lenvatinib was found to be linear in theconcentration range of 2 - 14ug / ml

Fig no: Calibration curve of Lenvatinib in methanol

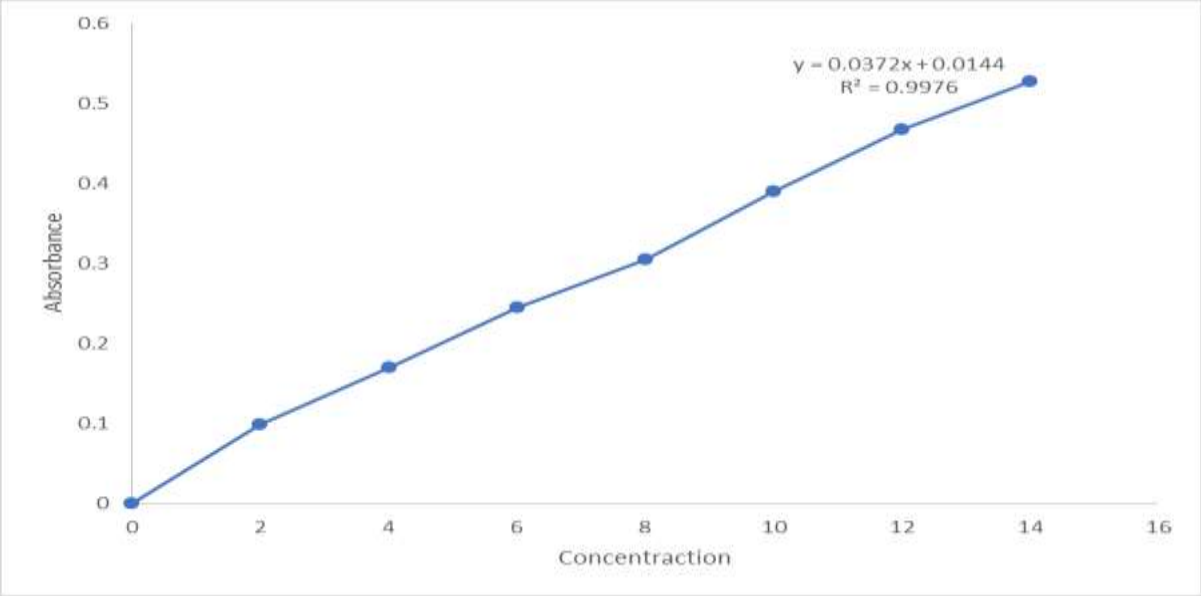


Table No. 11: Various Constants for Calibration Curve of Lenvatinib:

Parameter	Slope	Intercept	R^2
Value for Calibration curve in Liposomes	0.037	+ 0.014	0.997

4.2 Effect of Process Parameters on Liposome Preparation:

4.2.1. Speed of rotation:

Uneven film was observed at higher speed of rotation (150 rpm) during film formation. This may be due to improper distribution of heat at higher speed. During film formation, lower speed increases the time of contact of the film with the hot water in the water bath. At higher speed, since sufficient time is not available for the lipid to form film, liquid to gel transitions of the lipid does not occur. Based upon these observations, 75 rpm was considered optimum for formation of uniform film. But, during hydration of lipid film sufficient energy and vigorous shaking is required for the dried film to get hydrated and form liposomes, thus 100- 150 rpm was found adequate to form uniform liposomal dispersion.

4.2.2. The ratio and volume of solvent blend:

The choice of solvent is usually chloroform owing to its low boiling point (61.150C) and easy removal under vacuum leading to thin film deposition on the wall of the flask. Lenvatinib is soluble in DMSO and both the lipids (Cholesterol and Phosphatidylcholine) are soluble in chloroform. Thus, DMSO was used in combination with chloroform. Chloroform: DMSO 3:3 ratio was selected. This ratio gave an even, uniform film. When these ratio changes then entrapment and practical yield also changes and it showed decrement in practical yield as well as entrapment efficiency.

4.2.3. pH of the hydrating media:

According to the Handerson Hassel batch equation, pka is defined as the extent to which a drug is available in the ionized form at a given pH.

$$pH = pKa + \log (ionized/unionized) \quad (1.4)$$

Hence, the extent of ionization is influenced by the pKa of any drug. Thus, entrapment of any drug depends

on its pka to a considerable extent. Maximum lonization (pKa 90%) would hence accurate pH values one unit of the pka value. Phosphate buffer pH 7.4 was selected as it yielded uniform suspension with minimum sedimentation and maximum entrapment efficiency. Phase separation was observed towards acidic pH. Hence, further batches were prepared at pH value of 7

4.2.4 Optimization of Liposomal preparation by change in molarity of calcium chloride solution:

The calcium chloride solution is required for trapping method of Liposome formation to bind with liposomal formulation and form cigar like structure to that Liposomes. So, if the change in molarity of calcium chloride solution then it affects on practical yield as well as entrapment efficiency. But when we taken 0.1 M calcium chloride solution then that all batches showed better results in practical yield and entrapment efficiency.

4.3 Characterization of Lenvatinib Loaded Liposomes:

4.3.1. Physical Appearance:

The Liposomes suspension was found to be:

Table no. 12. Physical Appearance of prepared formulation:

Parameter	Observation
Appearance	Suspension
Color	Pale yellowish to White Creamy
Odor	Odorless

4.3.2. Practical yield:

Practical yield of prepared Liposomes were obtained in the range of 92.43±1.44 to 63.45±1.22 as shown in the table no. 13. The production yield of Liposomes decreased due to the improper concentration of cholesterol and lipid ratio, because it had some stickiness products.

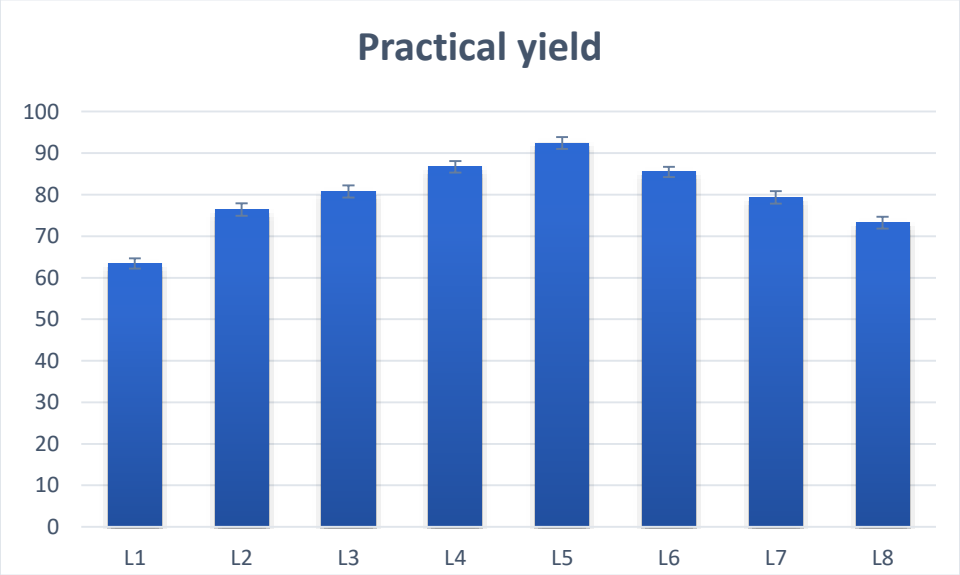


Table no. 13. Practical yield of prepared liposomes:

Batch code	% Practical yield
L1	**63.45 ± 1.22**
L2	**76.40 ± 1.52**
L3	**80.77 ± 1.45**
L4	**86.73 ± 1.38**
L5	**92.43 ± 1.44**
L6	**85.47 ± 1.25**
L7	**79.35 ± 1.51**
L8	**73.24 ± 1.41**

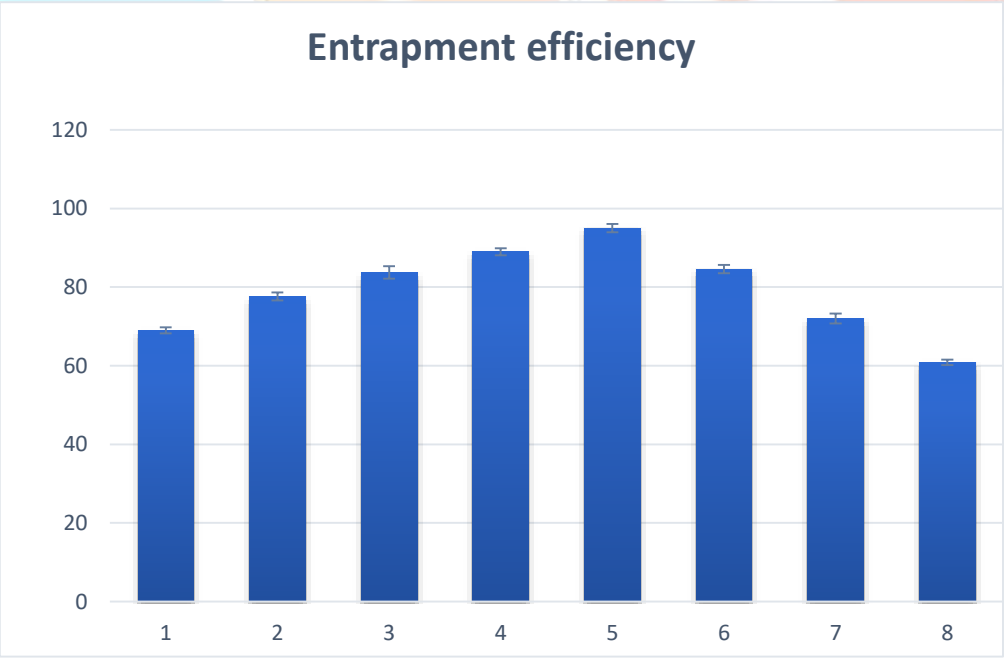
Each value is average of three separate determinations ± SD. 9.3.3.

Entrapment Efficiency:

Entrapment efficiency as shown in the Table No. 16 indicates that while changing the ratio of phospholipid and cholesterol, entrapment efficiency was got increased upto ration becomes equalbut later on it again decreases as ratio changes. The molarity of calcium chloride solution was also responsible for change in entrapment efficiency only the 0.1 M calcium chloride solution shows better results. The entrapment efficiency of Diosmin was observed that it depends on the concentration of cholesterol and lecithin as well as molarity of calcium chloride solution was used.

Table no.14 Entrapment Efficiency prepared liposomes:

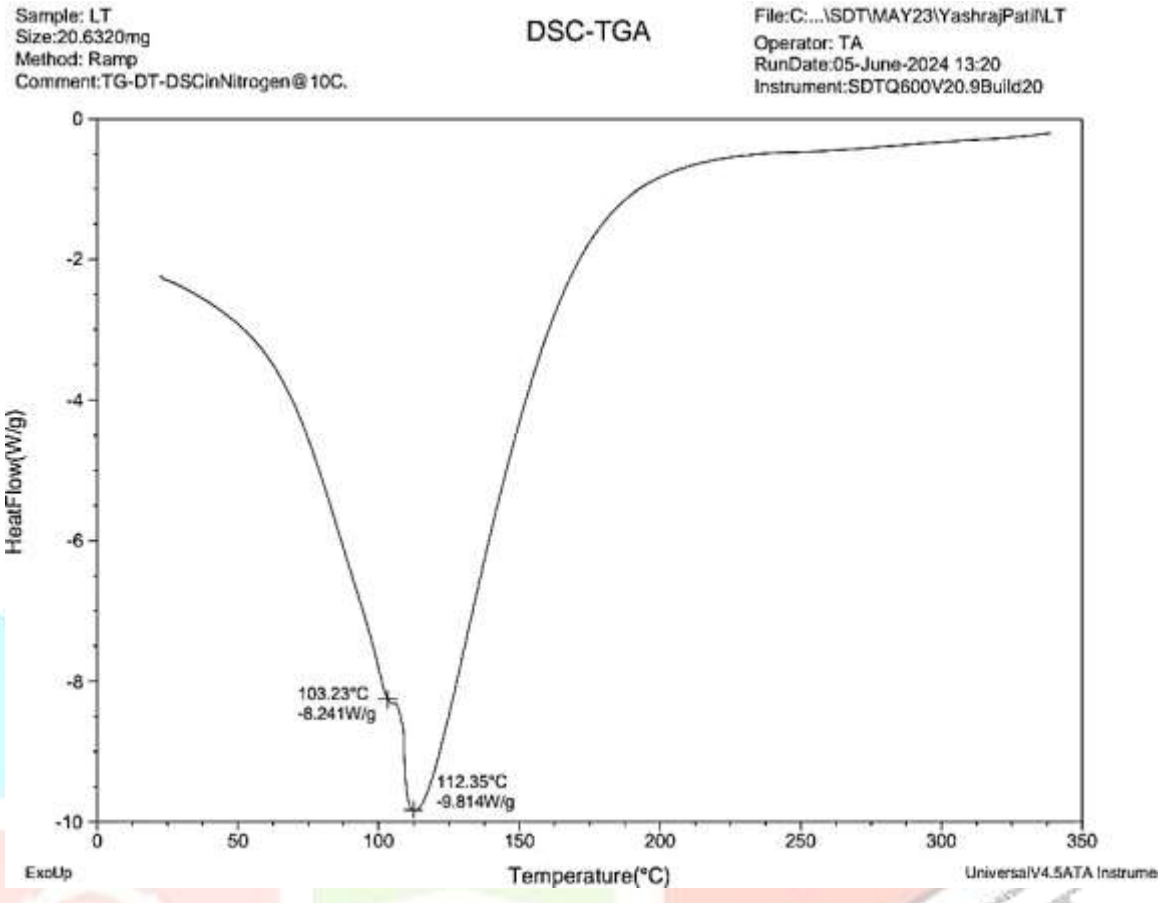
Batch code	Entrapment Efficiency (%)
L1	69.00 ± 0.80
L2	77.66 ± 1.02
L3	83.71 ± 1.60
L4	89.00 ± 0.90
L5	**95.00 ± 1.03**
L6	84.57 ± 1.06
L7	72.00 ± 1.27
L8	60.83 ± 0.70



4.3.4. Differential Scanning Calorimeter Analysis:

Diffraction Scanning Calorimeter (DSC) monitors heat effects associated with phase transition and chemical reaction occurs with a function of temperature. In a DSC the difference in heat flows to the pure drug Lenvatinib and a formulation of optimized batch M5at the same temperature and the difference in heat flow to the sample and a reference at the same temperature are recorded as a function of temperature. DSC analysis shows variation, change or comparison in study of tg and melting point of formulation or pharmaceutical excipients shows thermograms of Lenvatinib and nanocochleates optimize batch M5 and Differential Scanning Calorimetry enables the quantitative detection of all processes in whichenergy is required or produced Thermogram of prepared nanocochleates have been depictedin Fig. 25 and 26. In

the case of pure Lenvatinib a sharp endothermic peak was observed at 184°C, corresponding to the melting point of Lenvatinib. In the optimized nanocochleates batch endotherm observed at near about 117°C with the sharp appearance. It reveals that is no lipidemic transition during nanocochleates preparation of Lenvatinib.



CONCLUSION:

In conclusion, the integration of liposome-based delivery systems for lenvatinib presents a promising advancement in cancer therapy, particularly for hepatocellular carcinoma (HCC). The development of HA-modified lenvatinib-loaded micelles (HA@Len-M) demonstrated potential in improving the drug's solubility, bioavailability, and targeting efficiency. By focusing on the tumor microenvironment (TME) and the enhanced permeability and retention (EPR) effect, this novel delivery system addresses the limitations of traditional lenvatinib therapy, such as poor aqueous solubility and severe adverse reactions.

The study's findings highlight the benefits of using liposomal formulations to enhance the delivery and therapeutic efficacy of Lenvatinib. The HA@Len-M micelles effectively targeted CD44 proteins on H22 cell membranes, showcasing the potential for improved treatment outcomes through targeted drug delivery. The physicochemical properties and stability of the micelles further support their viability as a drug delivery system.

Overall, this investigation underscores the importance of innovative drug delivery systems in cancer treatment. Liposomal formulations, such as HA@Len-M, not only improve the solubility and stability of hydrophobic drugs like Lenvatinib but also enhance their therapeutic index by concentrating the drug at the tumor site and minimizing systemic exposure. Future research should continue to explore the optimization and clinical application of such systems to provide more effective and safer cancer therapies.

REFERANCE:

[1] Siegel, R.L.; Miller, K.D.; Jemal, A. Cancer statistics, 2018. CA Cancer J. Clin. 2018, 68, 7–30. [CrossRef] [PubMed]

[2] Massague, J.; Batlle, E.; Gomis, R.R. Understanding the molecular mechanisms driving metastasis. Mol. Oncol. 2017, 11, 3–4. [CrossRef] [PubMed]

[3] Saggar, J.K.; Yu, M.; Tan, Q.; Tannock, I.F. The tumor microenvironment and strategies to improve drug distribution. Front. Oncol. 2013, 3, 154. [CrossRef] [PubMed]

[4] Allen, T.M.; Cullis, P.R. Liposomal drug delivery systems: From concept to clinical applications. Adv. Drug. Deliv. Rev. 2013, 65, 36–48. [CrossRef] [PubMed]

[5] Maeda, H. Toward full understanding of the epr effect in primary and metastatic tumors as well as issues related to its heterogeneity. Adv. Drug Deliv. Rev. 2015, 91, 3–6. [CrossRef] [PubMed]

[6] Luo X, Ao S, Li C, Fang L, Chen L, Liu H, Li J, Zhou Y, Yin X, Wu C, Xi M. Enhanced delivery of lenvatinib by hyaluronic acid-polyglycerol-stearate self-assembled micelles to inhibit hepatocellular carcinoma in vitro. Journal of Drug Delivery Science and Technology. 2023 Sep 1;86:104631.

[7] Matsui J, Yamamoto Y, Funahashi Y, Tsuruoka A, Watanabe T, Wakabayashi T, Uenaka T, Asada M: E7080, a novel inhibitor that targets multiple kinases, has potent antitumor activities against stem cell factor producing human small cell lung cancer H146, based on angiogenesis inhibition. Int J Cancer. 2008 Feb 1;122(3):664-71. [Article]

[8] Glen H, Mason S, Patel H, Macleod K, Brunton VG: E7080, a multi-targeted tyrosine kinase inhibitor suppresses tumor cell migration and invasion. BMC Cancer. 2011 Jul 22; 11:309. doi: 10.1186/1471-2407-11-309. [Article]

[9] Scott LJ: Lenvatinib: first global approval. Drugs. 2015 Apr;75(5):553-60. doi: 10.1007/s40265-015-0383-0. [Article]

[10] Killock D: Neuroendocrine cancer: SELECT-lenvatinib in thyroid cancer. Nat Rev Clin Oncol. 2015 Apr;12(4):189. doi: 10.1038/nrclinonc.2015.30. Epub 2015 Feb 24. [Article]

[11] Bangham AD, Standish MM, Watkins JC. Diffusion of univalent ions across the lamellae of swollen phospholipids. J. Mol. Biol. 1965; 13(1): 238-252.

[12] Lachmann and Lieberman's. The theory and practice of industrial pharmacy, CBS Publication, New Delhi, fourth edition, 2013; 217-254.

[13] Kumar N. Jain A. K, Singh C. Agarwal K. Nema R. K.: Development, characterization and solubility study of solid dispersion of Terbinafine hydrochloride.

[14] Bandgar SA, Formulation and evaluation of prazosin hydrochloride loaded solid lipid nanoparticles, Journal of Drug Delivery and Therapeutics, 2018; 8(6-s):63-69.

[15] Bhise Kiran S, Naeem Mohammed, Formulation and development of fenofibrate loaded liposphere system, Journal of Drug Delivery & Therapeutics; 2013, 3(1), 1-10.

[16] Suraj R Wasankar, Syed M Faizi, Abhisek D Deshmukh. Formulation and development of liposomal gel for topical drug delivery system. IJPSR, 2012; 3(11): 4461-4474.

[17] Bhupendra Pradhan, Narendra Kumar, Suman Saha, Amit Roy: Liposome: Method of Preparation, Advantages, Evaluation and Its Application, J of App. Pharma. Res, 2015; 3(3), 1-8.

[18] Singh A, Nainwal P, Nanda D. Jain DA. Solubility Enhancement Study of Pioglitazone Using Solid Dispersion as Solubilization Technique. USID. 2011; 1 (2):95-100.

[19] Dash Tapaswi Rani. Liposome as a Potential Drug Delivery System: A Review. IRJP. 2013; 4 (1);6-12.

[20] Akshay Singha Roy. Sudipta Das, Arnab Samanta. Design, formulation and evaluation of liposome containing isoniazid. International Journal of Applied. Pharmaceutics, 2018; 10(2): 52-56.

[21] Henriksen I, Sande SA, Smistad G, Agren T. Karlsen J. In vitro evaluation of drug release kinetics from liposomes by fractional dialysis. Int. J. Pharm. 1995; 119: 231-8.