*https://doi.org/ 10.33472/AFJBS.6.9.2024.3661-3679*


# Valacyclovir hydrochloride liposomes: characterization of preformulation parameters, formulation development, and comparative evaluation using two approaches.

**A.A. SHAIKH, A.J. SAMUEL***

Department of Pharmaceutics, Allana College of Pharmacy, Azam Campus, Camp, Pune-411001, India

*Corresponding author email: *aneyjoice@azamcampus.org*

**Article History:Volume 6,Issue 9, 2024: Received: 28 Apr 2024:Accepted : 10 May 2024: doi: 10.33472/AFJBS.6.9.2024.3661-3679**

## ABSTRACT

Valacyclovir is a prodrug, an esterified version of aciclovir that has greater oral bioavailability than acyclovir. It is an antiviral drug that has been used to manage and treat various herpes infections. A liposome is the most acceptable and superior carrier that can encapsulate both hydrophilic and lipophilic drugs and protect them from degradation. Valacyclovir hydrochloride liposomes were prepared by two different methods viz: ether injection and the thin-film hydration method. The liposomes obtained were evaluated for percentage yield, vesicle size, and entrapment efficiency.

Since the thin-film hydration method yielded more liposomes with better vesicle size and entrapment efficiency than the ether injection method, the study continued with the formulation and evaluation of Valacyclovir hydrochloride liposomes by the thin-film hydration method. The drug encapsulation efficiency varied from 71 % to 87 %. In vitro drug release studies were carried out and formulation exhibited around 70% drug release in 3hrs.

**Keywords**: **Liposomes, Valacyclovir hydrochloride, Ethanol injection, Thin-film hydration.**

## INTRODUCTION

Valacyclovir Hydrochloride (VH) is rapidly converted to acyclovir which has demonstrated antiviral activity against herpes simplex virus types 1 (HSV-1) and 2 (HSV-2) and varicella-zoster virus (VZV) both in vitro and in vivo. After oral administration, valacyclovir hydrochloride is rapidly absorbed from the gastrointestinal tract. Valacyclovir is converted to acyclovir and L-valine by first-pass intestinal and/or hepatic metabolism. Peak plasma valacyclovir concentrations are generally less than 0.5 mcg/mL at all doses. The plasma elimination half-life of acyclovir typically averaged 2.5 to 3.3 hours in all studies of VALTREX in volunteers with normal renal function.

The pioneer of liposomes was Dr. Alec D. Bangham, a hematologist by profession. He discovered it in 1964. He added a negative stain to the phospholipid and observed it in the microscope. This phospholipid showed structural similarity to the cell membrane [1]. Liposomes are derived from Greek, where "Lipo" means fat and "somes" means body. The membrane of these phospholipids has two parts: the hydrophilic

portion which is the head and the hydrophobic portion which is the tail. The hydrophilic heads are aligned toward the water, and the tail is repelled by water.

The main aim of a drug delivery system is to provide a proper therapeutic action at the desired site in the body and maintain the drug concentration range. The nano formulation’s reduced size increases the surface area's ratio to volume and thus increases the bioavailability [2, 3, 4]. Thus, liposomes are nano formulations which fulfill this purpose.

Liposomes are vesicles that have homocentric phospholipids bilayer molecules. These phospholipids range from low molecular weight to high molecular weight and have been used in the preparation of liposomes. The water-soluble drugs are present in an aqueous compartment, whereas lipid-soluble drugs and amphiphilic drugs are present in a phospholipid bilayer. The liposomes containing the drugs may be administrated by many routes like oral inhalation, intravenous, local application, and ocular, and thus can be used to treat various diseases.

Multilamellar liposomes (MLV) sometimes vary from five hundred to 10,000 nm. Unilamellar liposomes can be referred to as tiny (SUL) and as giant (LUV): SUVs are sometimes smaller than 50nm, and LUVs are sometimes more giant than 50nm. The liposomes of extra-large size are referred to as giant liposomes (10,000-10, 00, 00 nm). They can be either unilamellar or multilamellar. The liposomes containing encapsulated vesicles are referred to as multi-vesicle liposomes. Their size ranges from 2,000-40,000 nm. LUVs with asymmetric phospholipids distribution within the bilayer are referred to as uneven liposomes.

Topical drug administration may be a localized drug delivery system anywhere within the body through ophthalmic, rectal, epithelial ducts, and skin as topical routes. Skin is one of the most accessible organs for topical administration and is the route of the topical drug delivery system [5, 6, 7]. The topical application of liposome vesicles has several benefits over the conventional dosage forms. Thanks to the bilayer composition and structure, they have been more practical and less cyanogenic than standard formulations. Liposomes are typically applied to the skin as liquids or gels. Hydrophilic polymers are considered to be suitable thickening agents for the topical application of liposomes in gel form. Liposome carriers, standard for their potential in topical drug delivery, are chosen to assist the transport of drug molecules within the skin layers. There are different methods of formulation of liposomes [8, 9, 10, 11]. In this study, the two methods of formulation of liposomes are discussed. They are the thin-film hydration method and the ethanol injection method.

## EXPERIMENTAL

### Materials:

Valacyclovir Hydrochloride was received as a gift sample from Dhamtek Pharma, Navi Mumbai. Soya lecithin and Cholesterol were purchased from Research Lab, Fine Chem Industries, Pune. All other chemicals and solvents used were of analytical reagent grade. Phosphate buffer 7.4 was freshly prepared with distilled water according to IP.

### Methods:

### Characterization of drug & excipients:

When an active pharmaceutical ingredient (API) is being characterized for use in a drug

product, its chemical and physical characteristics are usually the main focus.

**Organoleptic properties:**

The VH and excipient samples were taken and analyzed for organoleptic properties like state, color, odor, and appearance.

**Visual Solubility in various solvents:**

The excess VH dissolved in 10ml of different solvents like Distilled water, Methanol, Phosphate buffer (pH 7.4), and DMSO in the volumetric flasks. They were subjected to a linear motion shaker for 24 hrs. The solutions were observed visually for their clarity. The observed data of visual solubility has been depicted in Table No-3.

**Analytical characterization:**

Analytical analysis of bulk drug materials, intermediates, drug products, drug formulations, contaminants and degradation products, and biological samples containing the pharmaceuticals and their metabolites is crucial in the field of pharmaceutical research. There is a wide range of analytical techniques available to fulfill sample characterization needs. To characterize the drug analytically for λmax and linearity in phosphate buffer pH 7.4, UV visible spectroscopy and FTIR spectroscopy were used to examine the functional groups.

**Drug excipient compatibility:**

The majority of solid dosage forms are made up of excipients, which operate as diluents to enable the creation of tablets with the right size and coatings to shield the tablet from the drug's unwanted organoleptic properties. When a drug substance is reactive, solid-state reactions in the dosage form can happen, and they can be amplified by the

drug ingredient's physical and chemical interactions with excipients. Sometimes excipients encourage the breakdown of the drug material rather than interacting chemically.

The compatibility of medications and excipients was investigated using FTIR. The spectra of pure valacyclovir, soy lecithin, and cholesterol, as well as their physical combination, were recorded using the potassium bromide (KBr) disc technique. Between 400 and 4000 cm-1 was the measured range of the infrared light spectrum.

Figures 3 and 4 depict the IR spectra of a pure medication and combination.

**Formulation of liposomes for method selection**

**Thin-film hydration method [12, 13]**

Valacyclovir hydrochloride liposomes were prepared by using the thin-film hydration method. Drug, soya lecithin, and Cholesterol were taken in different concentrations and dissolved in chloroform and methanol. This mixture was transferred into a clean round bottom flask. Chloroform and methanol were added in 1:1 ratio, and the flask was fixed to the rotary evaporator. The solvent was evaporated by maintaining the temperature at 30-40 °C under a vacuum at 50 pm. It forms a dry, thin film and is vortexed at room temperature for 20 min., forming a milky white suspension. Then the dry film of the liposomes was rehydrated with Phosphate buffer, pH 7.4. For this, 20 ml of phosphate buffer was added to the round bottom flask and vortexed at 20 rpm for 20 minutes. Thus, it gives rise to the nanoparticles that are liposomes.

**Ethanol injection method [14, 15, 16]**

Soya lecithin and Cholesterol were taken in the ratio of 50:30 was dissolved in absolute

ethanol. The solution was heated at 55 ºC, which is the temperature above the gel to liquid crystal phase transition temperature. The heating is done to improve the miscibility of the phospholipid in the organic solvent. Proceeding this, 1ml of the ethanolic solution was loaded in a 21-gauge needle and injected at the rate of 1ml/min in 16ml of water containing 200mg of drug kept at 55 ºC and magnetically stirred at 300rpm. The rate of injection was maintained constant with the help of an electronic dynamometer. Thus, the formation of instant liposomes takes place as soon as the organic phase comes in contact with the aqueous phase. The liposomal suspension was kept stirring for 30 min. at room temperature. The final ethanol concentration in the raw liposomal dispersion was 6% v/v. To obtain the dispersions with low ethanol content, namely 0.1% and 1% v/v, instead after liposome preparation, the residual solvent was partially removed by a rotary evaporator.

**Preliminary evaluation for method selection**

**Percentage yield:**

The percentage yield is crucial to the production process. The % ratio of the actual yield to the theoretical yield is known as the percentage yield, and it is used to gauge how effective a chemical reaction is.

**Vesicle size measurement:**

The produced liposomes were examined to see how the vesicles formed and how distinct the distributed vesicles were. Using an optical microscope, the liposomes' particle size was ascertained. Particle size was determined as mean diameter and is based on direct observation under the microscope. The procedure includes the

calibration of an eye-piece micrometer and the measurement of globule size.

**Entrapment efficiency:**

The percentage of medication that is successfully encapsulated into liposomes is known as encapsulation efficiency. It is determined by measuring the amount of integrated material found in the formulation over the concentration that was first utilized to create it. One of the most crucial factors in the composition of liposomes is encapsulation efficiency, which indicates the concentration of active substances.

**Formulation of VH-loaded liposomal dispersions:**

Based on the preliminary evaluation, the liposomes were prepared using the thin film hydration method for further study. The procedure adopted was as mentioned before with different ratios of cholesterol and soya lecithin. The obtained VH liposomes were characterized by the following parameters.

**Characterization of liposomes [13, 14]**

**Drug content:**

The concentration of liposome valacyclovir was determined by UV spectrophotometric analysis. After the proper dilution, the absorbance was measured using a UV spectrophotometer at 252nm against a blank to determine the maximum amount of medication present.

**Size & Zeta Potential measurement:**

Both diffused and discrete vesicle production was monitored in the liposome-preparing batches. The motic microscope was used to determine the particle size. By taking the images and calculating the mean diameter on the computer, the particle size was

discovered. Using a Malvern Zetasizer, the charge on the drug-loaded vesicle surface was measured.

**Entrapment efficiency:**

A lab centrifuge (2C4BL, Remi) was used to centrifuge 10 ml of liposomal suspension for 90 minutes at 4000 rpm. To extract the medicine that was not entrapped, the supernatant was carefully removed, and the absorbance at 252 nm was measured. After diluting the sediment in the tube with PBS 7.4, the absorbance of this solution was measured at 252 nm. The total amount of drug in the dispersion was determined by adding the amounts of drug in the sediment and supernatant. This allowed for the computation of the drug's entrapment efficiency in the dispersion.

**In vitro drug release:**

In the Invitro release study, the Franz diffusion cell is used. Goat skin is used as a membrane for drug release. The ear pinna is mainly used for drug release studies. The donor compartment is filled with VH Liposomal suspension. In the receptor compartment, 20 ml of phosphate buffer (pH 7.4) is used. This study was conducted at room temperature. A magnetic stirrer was used throughout the entire procedure, stirring the solution at 100 rpm. 5 ml aliquots are withdrawn at a fixed time interval. At the end of the procedure, an equal amount of buffer in the cell is replaced. After the withdrawal, the sample is analyzed in a UV Spectrophotometer with a luminosity of 252 nm.

**RESULTS AND DISCUSSION**

**Organoleptic properties of the drug-**

**Table 1 Organoleptic Properties of Drug and Excipients**

| Physical characterization | **State** | **Color** | **Odor** | **Appearance** |
|---|---|---|---|---|
| Drug (V.H.) | Solid | White | Odorless | Powder |
| Cholesterol | Solid | White | Odorless | Crystalline powder |
| Soya Lecithin | Semisolid | Yellowish Brown | Pungent | Waxy |

**Table 2 List of instruments**

| **Sr. No** | **Name of the Instrument** | **Model No** | **Manufacturer** |
|---|---|---|---|
| **1** | Double Beam UV Spectrophotometer | V-630 | Jasco Corporation, Tokyo, Japan |
| **2** | Fourier Transmittance Infrared spectrophotometer | FTIR 4100 | Jasco Corporation, Tokyo, Japan |

**Visual Solubility in various solvents:**

The Solubility of valacyclovir hydrochloride was estimated in different solvents like distilled water, methanol, phosphate buffer 7.4, DMSO, and ethanol, and it is depicted in Table 3 below.

**Table 3 Visual Solubility of VH in Different Solvent Systems**

| S. No. | **Solvents** | **Solubility of VH** |
|---|---|---|
| 1 | Distilled water | Soluble |
| 2 | Methanol | Slightly soluble |
| 3 | Phosphate buffer pH 7.4 | Soluble |
| 4 | DMSO | Soluble |
| 5 | Ethanol | Slightly soluble |

**Analytical characterization**

**Determination of λ max in phosphate buffer 7.4**

After preparing the stock solution, the λ max of valacyclovir hydrochloride in phosphate buffer 7.4 was found to be 252 nm.

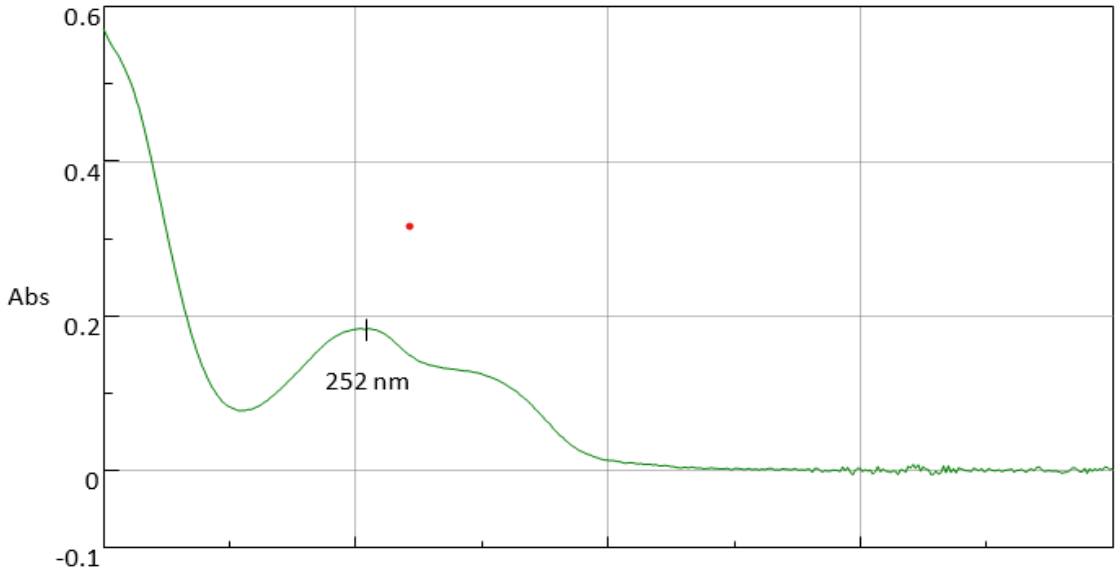


**Figure-1 Determination of λ max in PBS**

**Calibration Curve of Valacyclovir Hydrochloride in PBS 7.4**

The standard calibration curve of V.H. was plotted, and the points showed linearity with an $R^2$ value of 0.994, which shows that it obeys the Beer Lamberts law in the range of 5-25ug/mL.

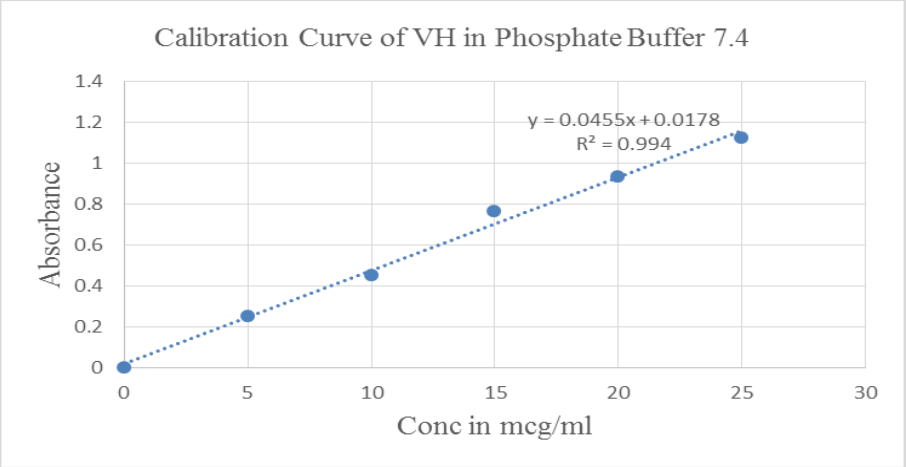


**Figure-2 Calibration Curve of VH in Phosphate Buffer 7.4**

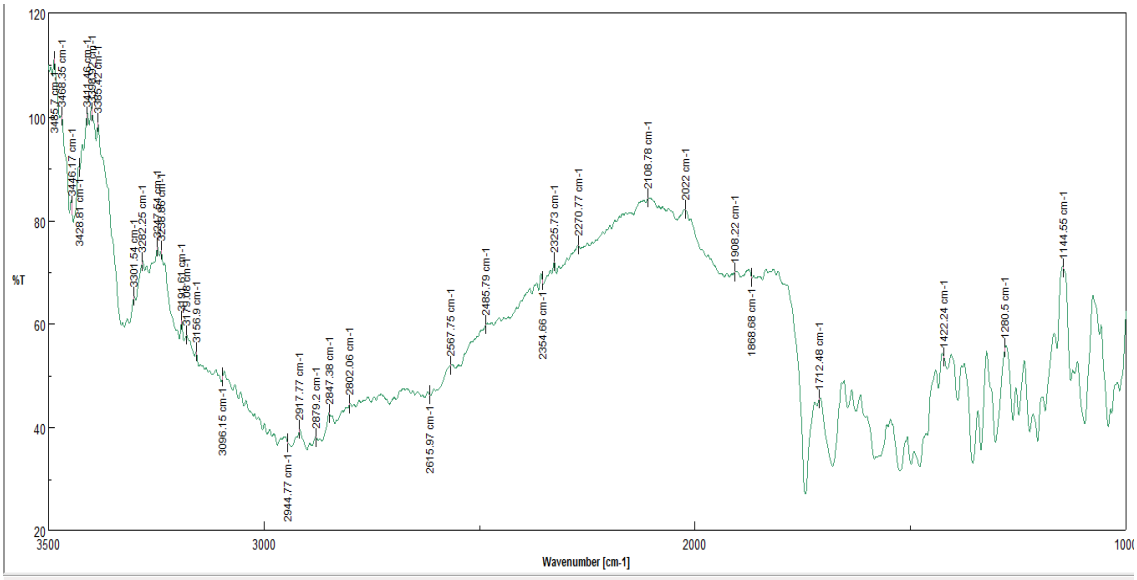


**Figure-3 FTIR of Valacyclovir hydrochloride**

**Drug excipient compatibility:**

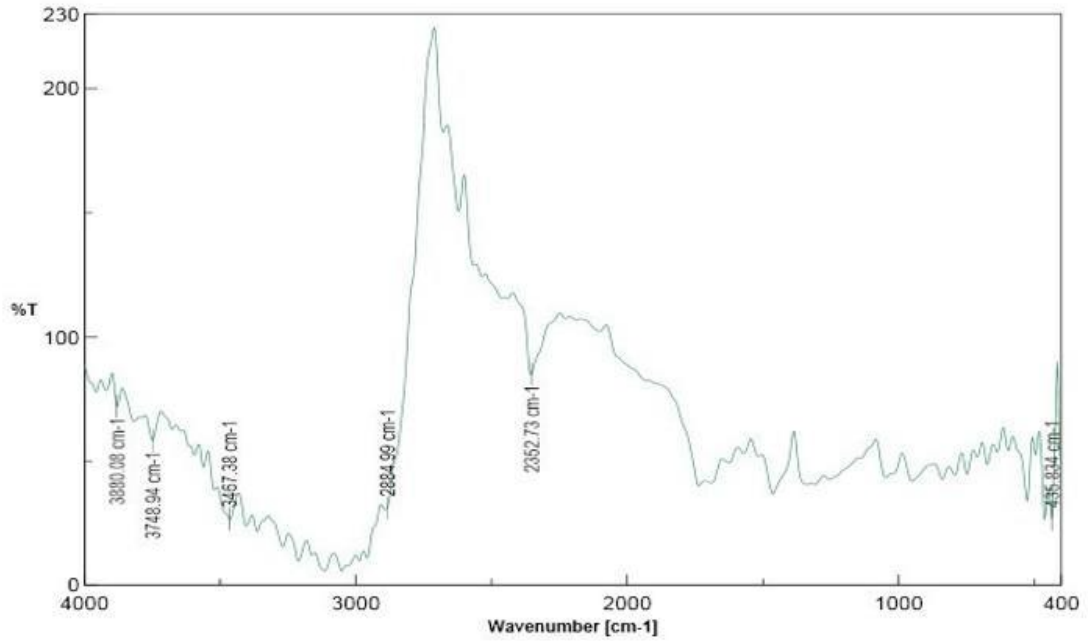


**Figure-4 FTIR of Drug and excipients combination**

**Table 4- Comparative evaluation of Liposomes**

| **Method used** | **Yield of formulation (mg)** | **% Yield** | **Vesicle size(nm)** | **Entrapment Efficiency (%)** |
|---|---|---|---|---|
| **Thin-film hydration** | 870 mg | 87% | 4.98±0.42 | 86.62±0.81 |
| **Ethanol injection** | 580 mg | 58% | 8.24±0.15 | 72.15±0.56 |

Results are ±SD, n=3

**Table 5- Formulation of VH Liposomes by Thin Film Hydration Method**

| Formulation code | Cholesterol: Soya Lecithin ratio |
|---|---|
| F1 | 1:2 |
| F2 | 1:3 |
| F3 | 1:4 |
| F4 | 2:2 |
| F5 | 2:3 |
| F6 | 2:4 |
| F7 | 3:2 |
| F8 | 3:3 |
| F9 | 3:4 |

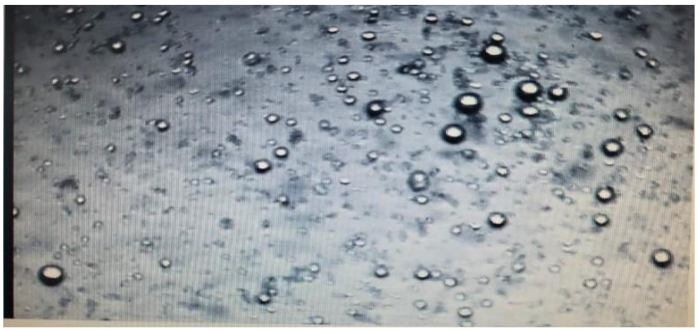

**Figure-5 VH liposomes**

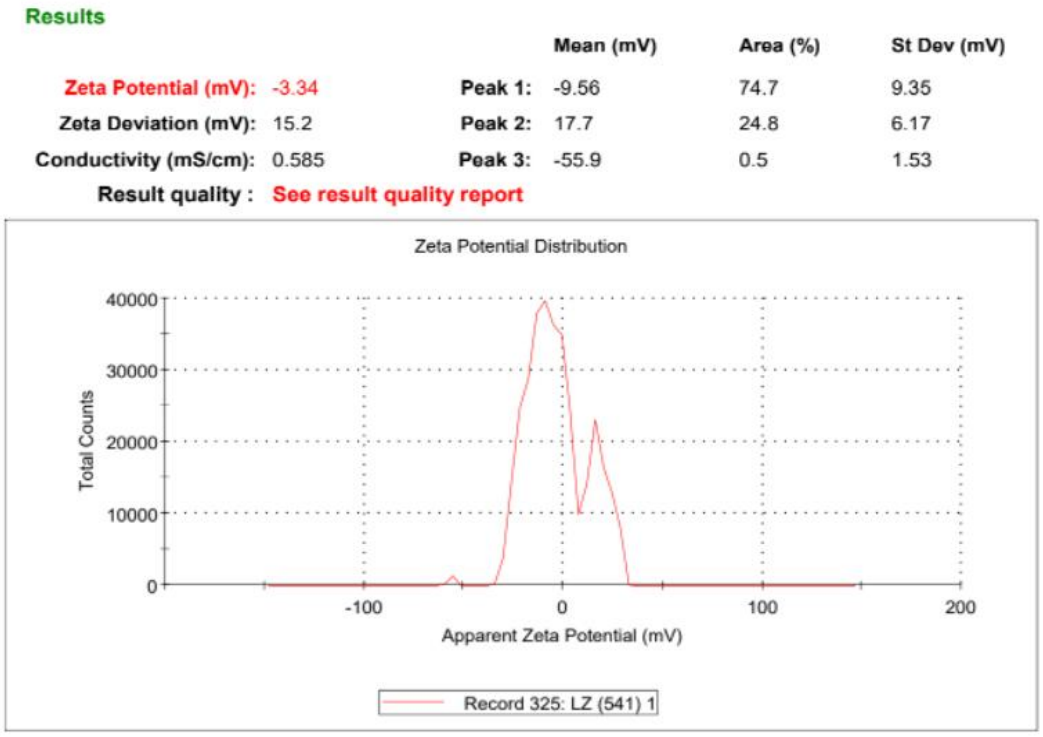


**Figure-6 Zeta Potential measurement using Zetasizer**

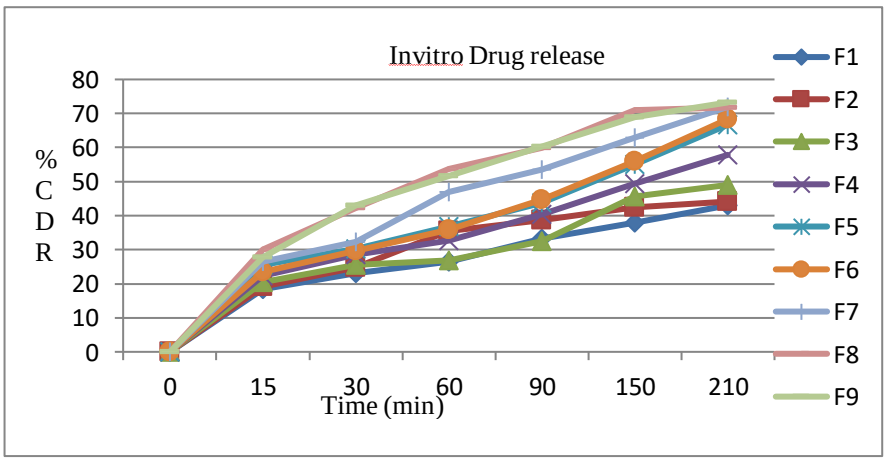


**Figure-7 In vitro Drug release from VH Liposomes**

The preformulation studies for organoleptic properties such as color, odor, and appearance were done, and the solubility test was performed. The U.V. Spectroscopy results showed that the absorption maxima complied with the standards. The preformulation study for drug excipients compatibility study by FTIR showed no drug

interaction between drug and excipients. Soya lecithin and Cholesterol as excipients were used as liposomes prepared by the ethanol injection method and the thin-film hydration method. The practical yield of both methods was estimated, which indicates the thin film hydration method showed a higher yield.

## CONCLUSIONS

The comparative evaluation of liposomes prepared by the thin film and ether injection methods revealed that the thin film hydration method yielded superior results. This method demonstrated higher yield, enhanced encapsulation efficiency, and vesicle size with better stability compared to the ether injection method. These findings underscore the importance of method selection in liposome preparation with each method offering distinct advantages and limitations. Further research is warranted to optimize liposome preparation techniques for specific applications. In conclusion, for optimal VH liposome preparation, the thin film hydration method emerges as a preferred choice to the ether injection method.

## ACKNOWLEDGMENTS

The authors are thankful to Allana College of Pharmacy for providing an opportunity to conduct the research above.

## CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

**REFERENCES**

1. Hadis Daraee, Ali Etemadi, Mohammed Kouhi, Samira Alimirzalu & Abolfazl Akbarzadeh, 44(1): 381–391(2016); doi: 10.3109/21691401.2014.953633.
2. Ling H, Luoma JT and Hilleman D, 4(2): 47-55 (2013); doi: 10.4021/cr270w.
3. Akbarzadeh A, Samiei M and Davaran S, Nanoscale Res Lett 7(1): 144 (2012); doi.org/10.1186/1556-276X-7-144
4. Mandal S, Vishvakarma P. Nanoemulgel: A Smarter Topical Lipidic Emulsion-based Nanocarrier. Indian J of Pharmaceutical Education and Research. 2023;57(3s):s481-s498.
5. Mandal S, Jaiswal DV, Shiva K. A review on marketed Carica papaya leaf extract (CPLE) supplements for the treatment of dengue fever with thrombocytopenia and its drawback. International Journal of Pharmaceutical Research. 2020 Jul;12(3).
6. Bhandari S, Chauhan B, Gupta N, et al. Translational Implications of Neuronal Dopamine D3 Receptors for Preclinical Research and Cns Disorders. African J Biol Sci (South Africa). 2024;6(8):128-140. doi:10.33472/AFJBS.6.8.2024.128-140
7. Tripathi A, Gupta N, Chauhan B, et al. Investigation of the structural and functional properties of starch-g-poly (acrylic acid) hydrogels reinforced with cellulose nanofibers for cu2+ ion adsorption. African J Biol Sci (South Africa). 2024;6(8): 144-153, doi:10.33472/AFJBS.6.8.2024.141-153

8. Mandal S, Bhumika K, Kumar M, Hak J, Vishvakarma P, Sharma UK. A Novel Approach on Micro Sponges Drug Delivery System: Method of Preparations, Application, and its Future Prospective. Indian J of Pharmaceutical Education and Research. 2024;58(1):45-63.
9. Mishra, N., Alagusundaram, M., Sinha, A., Jain, A. V., Kenia, H., Mandal, S., & Sharma, M. (2024). Analytical Method, Development and Validation for Evaluating Repaglinide Efficacy in Type Ii Diabetes Mellitus Management: a Pharmaceutical Perspective. Community Practitioner, 21(2), 29–37. https://doi.org/10.5281/zenodo.10642768
10. Singh, M., Aparna, T. N., Vasanthi, S., Mandal, S., Nemade, L. S., Bali, S., & Kar, N. R. (2024). Enhancement and Evaluation of Soursop (Annona Muricata L.) Leaf Extract in Nanoemulgel: a Comprehensive Study Investigating Its Optimized Formulation and Anti-Acne Potential Against Propionibacterium Acnes, Staphylococcus Aureus, and Staphylococcus Epidermidis Bacteria. Community Practitioner, 21(1), 102–115. https://doi.org/10.5281/zenodo.10570746
11. Raheem, Ahmad, Abbas, Dakhil, International J Res Ayurveda Pharm, 4(5); 742-46(2013); DOI: 10.7897/2277-4343.04524
12. Umbarkar M, Thakare S, Surushe T, Giri A, Chopade V, 11(1):72-76 (2021); DOI: http://dx.doi.org/10.22270/jddt.v11i1.4677.
13. Amin SG, Shah DA and Dave RH:. Int J Pharm Sci & Res 9(9): 3621-37 (2018); doi: 10.13040/IJPSR.0975-8232.9(9).3621-37.

14. Charcosset C., Juban A., Valour J.-P., Urbaniak S., Fessi H, Chem. Eng. Res. Des. 94, 508–515 (2015); DOI: 10.1016/j.cherd.2014.09.008.

15. Yoshie Maitani, Hiroshi Soeda, Wan Jumping, and Kozo Takayama, Journal of liposome research 11(1):115-25 (2001); doi: 10.1081/LPR-100103174.

16. Ahmed Gouda, Omar S. Sakr, Maha Nasr, Omaima Sammour, Journal of drug delivery science and technology: 6 (2021); https://doi.org/10.1016/j.jddst.2020.102174