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# FORMULATION AND EVALUATION OF LIPOSOMAL GEL OF ACYCLOVIR

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## ABSTRACT

Acyclovir is a synthetic purine nucleoside analogue with *in vitro* and *in vivo* inhibitory activity against human herpes Viruses, including Herpes Simplex Virus (HSV) types 1 and 2, Varicella Zoster Virus (VZV), Epstein Barr Virus (EBV) and Cytomegalovirus. Topical dosage forms provide relatively consistent drug levels for prolonged periods and avoid gastric irritation, as well as the other typical side effects of oral administration. The aim of the present study was to formulate and evaluate liposomal gel of acyclovir. In this research work liposomal gel of acyclovir were developed for topical application. Acyclovir liposomes were fabricated by thin film hydration technique. Bilayer composition of liposomal vesicles was optimized. The quantities of lecithin and cholesterol were changed to enhance the encapsulation of the drug. Prepared liposomes of acyclovir were incorporated into a gel using carbopol 941. The developed formulation was evaluated for drug entrapment efficiency, morphology, in-vitro drug release, drug Content etc. Formulation $F_3$(Lecithin: Drug: Cholesterol 100:50:20) was selected as optimized formulation and all the parameters viz. Entrapment Efficiency, Drug Content, Drug Release were found better.

## INTRODUCTION:

Liposomes are small artificial vesicles of spherical shape that can be created from cholesterol and non-toxic phospholipids[1]. Due to their size, hydrophobic and hydrophilic character (besides biocompatibility), liposomes are promising systems for drug delivery. Liposome properties differ considerably with lipid composition, surface charge, size, and the method of preparation[2]. Liposomes acts as drug carrier and releases the drug with a control rate[3]. Liposomes are extensively used carriers for numerous molecules in cosmetic and pharmaceutical Industries. Additionally, food and farming industries have extensively studied the use of liposome encapsulation to grow delivery systems that can entrap unstable compounds and shield their functionality[4].Liposomes can trap both hydrophobic and hydrophilic compounds, avoid decomposition of the entrapped combinations, and release the entrapped at designated targets. Because of their biocompatibility, biodegradability, low toxicity, and aptitude to trap both hydrophilic and lipophilic drugs and simplify site-specific drug delivery to tumor tissues[5].

Topical drug delivery is an attractive route for local and systemic treatment[8]. The delivery of drugs onto the skin is recognized as an effective means of therapy for local dermatologic diseases. Acyclovir is a guanosine analog that acts as an antimetabolite[9]. Viruses are especially susceptible. Used especially against herpes. In cell culture, acyclovir has the greatest antiviral activity against herpes simplex viruses, types 1 and 2 etc[10]. The mechanism of action of acyclovir is converted to its triphosphate form, acyclovir triphosphate (ACV-TP), Which Completely inhibits viral DNA polymerase, incorporates into and terminates the growing viral DNA chain, and inactivates the viral DNA polymerase[11]. This liposomal gel of acyclovir is used to treat "cold sores/fever blisters" (herpes labialis). It can speed up healing of the sores and decrease symptoms (such as tingling, pain, burning, itching).

## MATERIALS

Acyclovir was purchased from KP Laboratories Ltd., Hyderabad, India. Cholesterol, soya lecithin and all other chemicals used were laboratory reagent grade.

## METHODS

### Spectrophotometric determination of acyclovir

An ultraviolet Spectrophotometric method was used for estimation of acyclovir. The concentration was scanned over a range of 400-200 nm, resulted in a peak at 254 nm. The 254 nm was taken as absorption maxima for acyclovir. The standard graph and whole analysis was performed in phosphate buffer pH 6.8 solution.

### Preparation of standard stock solution

100mg of standard sample of acyclovir was dissolved in phosphate buffer PH 6.8 in 100 ml volumetric flask to get concentration 1mg/ml. Then take 10ml from this solution and dissolve it into 100 ml of phosphate buffer pH 6.8.The concentration of acyclovir in this second stock solution is 100ug/ml. Series of dilution prepared by transferring 0.2,0.4,0.6,0.8,1.0 ml into a 10ml volumetric flask. Then make up the volume up to 10 ml with phosphate buffer pH 6.8[19].The final concentration of acyclovir in these solutions are 2,4,6,8,10 ug/ml.

### Method of determining calibration curve of acyclovir

Absorbance of the final diluted solution is measured in a UV Spectrophotometer at 254 nm against phosphate buffer pH 6.8 as blank. The absorbance so obtained was tabulated and standard curve was plotted.

### Preparation of Liposomes

Liposomes were prepared by thin film method. Soya lecithin, cholesterol, vitamin E (as antioxidant) and 50mg acyclovir were dissolved in 10ml chloroform. The quantities of lecithin and cholesterol were changed to enhance loading drug in liposomes. Then the mixture was evaporated in a rotary evaporator at 50 rpm for 1hr.Then it was hydrated with phosphate buffer saline Ph 7.4 for 30 min[16 19]. Different ratios of all ingredients are given below in table (1).

### Preparation of Small Unilamellar Vesicle Liposome

The liposomes Prepared are multi lamellar vesicle (MLV). Small unilamellar vesicle (SVV) liposomes were obtained by sonication of MLV liposomes in an ultrasonic bath for 30 min at room temperature.

### Preparation of carbopol gel

Carbapol 941 (aqueous 1%) was added slowly to a phosphate buffer saline

solution (pH 7.4), under constant stirring. For gel preservation, methyl paraben (0.2%) was added. Then triethanolamine was added for achieving neutral pH and clearing of the gels. After addition of the full amount of solid material, the gels were allowed to swell under moderate stirring.

### Incorporation of liposome into carbopol gel

Liposomes are compatible with viscosity-increasing agents, e.g., methyl cellulose and carbopol. To provide suitable vehicles for topical application, liposomes can be distributed uniformly by incorporation into carbopol gel and can preserve its structure. Liposomal gel formulation was prepared by mixing the liposomal dispersion with the gels in the ratio of 1:5 (w/w) (liposomal dispersion/gel) [19 20].

### Drug-excipients compatibility studies

### Fourier transforms infrared (FTIR) spectroscopy

FT-IR studies were carried out on individual samples of cholesterol, acyclovir and optimized formulation of liposomal gel ($F_3$). The samples were mixed with IR grade KBr in the ratio of 1:100 and compressed using a hydraulic press under a pressure of 15000 lb. The pellets were scanned in an inert atmosphere over a wave number range of 4000-400 $cm^{-1}$.

## EVALUATION TESTS

### Determination of acyclovir entrapment

The liposomal suspension was centrifuged at 3000 rpm for 3-4 hrs. The Supernatant was removed and the liposomes were disrupted with ethanol 70% and the quantity of drug was measured using a Spectrophotometer at 254 nm.

Entrapment Efficiency = Drug Entrapped/ Total Drug X100

### Microscopy

Liposomes were examined by optical microscope to determine the shape and lamellarity of vesicles (magnification x200).

### Morphology

The particle size and morphology of the prepared liposomes was done by the scanning electron microscopy (SEM) at an accelerating voltage of 10kV. 9.3mm X 100SE. The morphology of the liposomes was also analyzed by Light Microscope.

**Drug Content:** 1 g of the prepared liposomal gel were mixed with 100ml of suitable solvent aliquots of which different concentration were prepared by suitable dilutions after filtering the stock solution and absorbance was measured drug content was calculated using the equation which was obtained by linear regression analysis of calibration curve.

### *In-Vitro* Drug Release Study

Studies of the drug release diffusion from liposomal system are directed toward the approaches that are relevant to the in vivo condition. In vitro diffusion studies were carried out using Franz diffusion. The lower cell reservoir and the glass cell top containing the sample and secured in place with containing the sample and secured in place with a pinch clamp the receiving compartment has volume of 15ml the system was maintained at $37\pm 0.5^0$ c by magnetic heater resulting in a membrane surface temperature of $32^0$ c a sample was placed evenly on the surface of the membrane in the donor compartment. 2 ml of receptor fluid were withdrawn from the receiving compartment at 30, 60, 90, 120,150,180,210,240 mins and replaced with 2 ml of fresh phosphate buffer saline pH 6.8 solution. Samples were assayed spectrophotometrically for drug content at 254 nm.

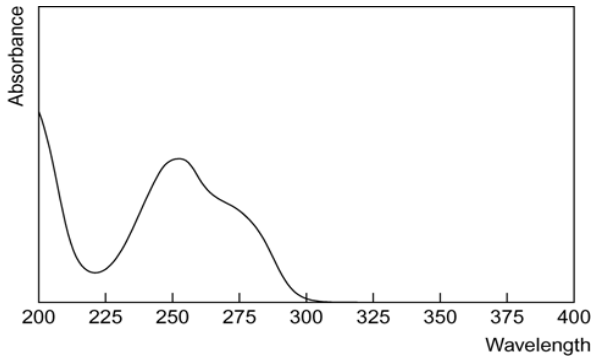



**Figure No.1: Absorption spectrum of acyclovir**

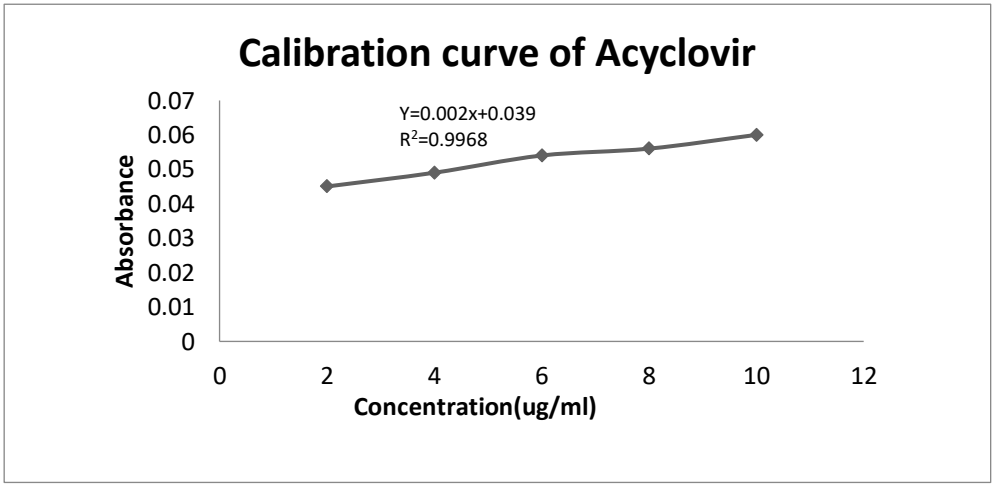



**Figure No.5: Standard calibration curve in phosphate buffer pH 6.8 at 254 nm**

**Table (1): Formulation with different Ratio**

| Formulation No | Compositions ratio(mg) (lecithin: drug: cholesterol) |
|---|---|
| F1 | 100:50:5 |
| F2 | 100:50:10 |
| F3 | 100:50:20 |
| F4 | 100:50:40 |
| F5 | 100:50:60 |

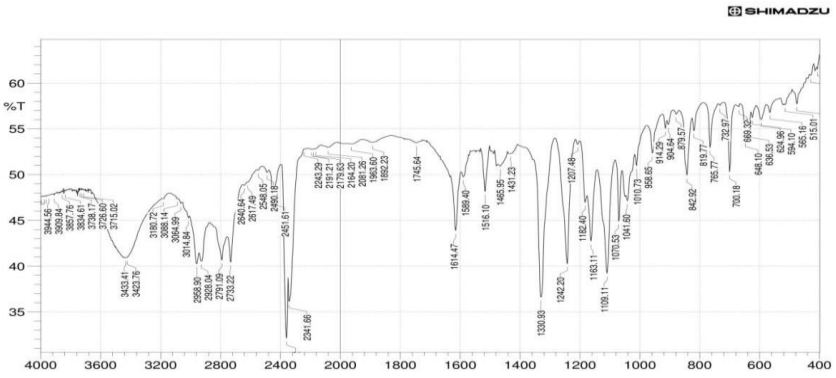



**Figure No.2: FTIR spectrum of acyclovir**

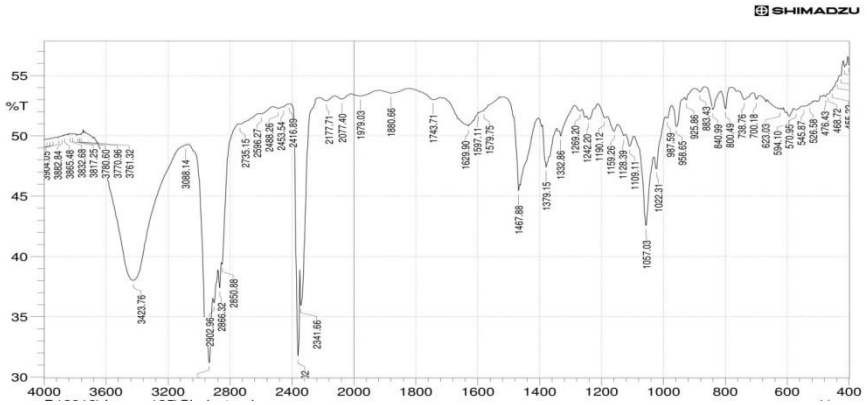



**Figure No.3: FTIR spectrum of cholesterol**

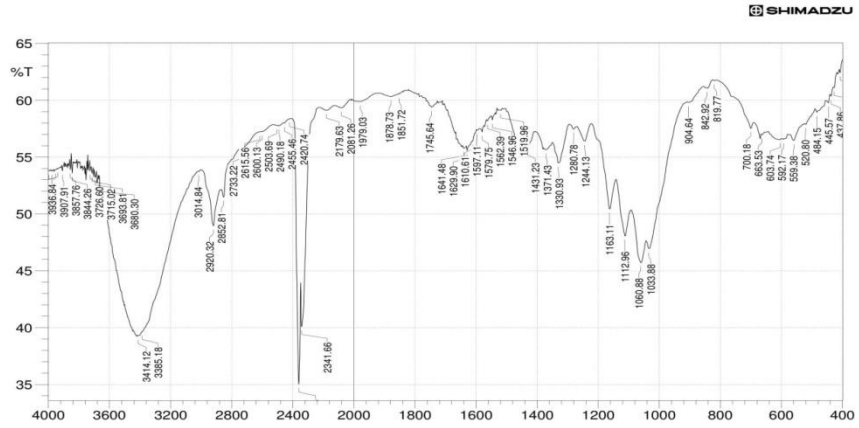
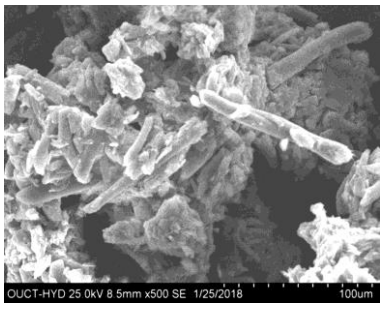



**Figure No.4: FTIR spectrum of formulation**



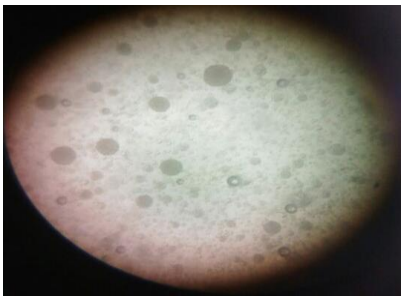



**Figure No.6: SEM image of acyclovir liposomes Figure No.7: Light microscopic image of Liposomes ($F_3$)**

**Table (2): Drug Entrapment Efficiency**

| Formulation No | Entrapment Efficiency (%) |
|---|---|
| $F_1$ | 34.68 |
| $F_2$ | 62.66 |
| $F_3$ | 78.30 |
| $F_4$ | 49.51 |
| $F_5$ | 51.33 |

**Table (3): Drug content**

| Formulation No | Drug content in % |
|---|---|
| $F_1$ | 56.92 |
| $F_2$ | 72.56 |
| $F_3$ | 91.33 |
| $F_4$ | 62.92 |
| $F_5$ | 68.66 |

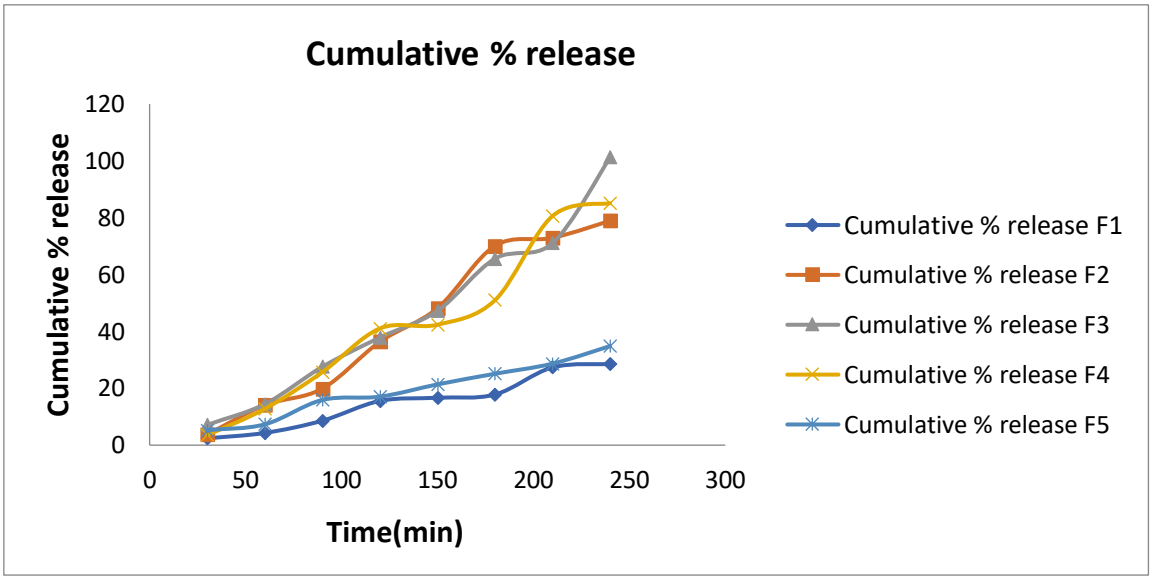



**Figure No.7: Comparative Diffusion Profile Data of all Formulation**

## RESULTS AND DISCUSSION

### Spectrophotometric determination of Acyclovir

The absorption spectrum of pure drug was scanned between 200-400 nm with 10 ug/ml concentration prepared in phosphate buffer pH 6.8 the absorption spectrum of resulting solution, when observed between 208-329 nm, exhibits maxima about 254 nm these peaks are similar to that given in standards.

**Development of calibration curve :** The calibration curve for Acyclovir was prepared in phosphate buffer solution pH 6.8.

**Formulation of liposomes:** Liposomes were prepared by using different concentration ratio of lecithin, drug and cholesterol.

**Preparation of small unilamellar vesicle liposomes:** Small unilamellar vesicle liposomes were prepared by using bath-sonicator.

**Drug-excipients compatibility studies:**

**FTIR Studies:** Drug and polymers identified by FTIR spectroscopy. The FTIR spectrum given below show that the peaks of the drug, polymer and optimized formulation[12].

FTIR Study showed that there was no major change in position of peaks obtained in alone and formulation, which shows that there was no interaction between drug and polymers.

## EVALUATION TESTS

**Microscopy:** Liposomes were examined by optical Microscope to determine the shape and lamellarity of vesicles.

**Morphology:** Morphology of the vesicles was performed with the help of a Scanning Electron Microscope (SEM).

**Drug Entrapment Efficiency of Acyclovir**

The drug Entrapment efficiency of the formulations ($F_1$-$F_5$) was estimated and the results were in the range of 34-78%.The drug Entrapment determination also showed that the drug was uniformly distributed throughout the preparation.

**Drug content:** The drug content of different formulation was in range 56-91% as shown in table (3).The maximum drug content was found in $F_3$.

***In vitro* drug release study:** Cumulative % release of different prepared formulations was calculated by using Franz diffusion cell. In vitro drug release studies of all the formulations of liposomal gels were carried out in phosphate buffer saline pH 6.8 solution. It was observed that ratio of lecithin, drug, cholesterol, influences the drug release pattern. Formulation with different ratio($F_3$) showed high release of drug when compared to formulations with other ratio($F_1$, $F_2$,$F_4$, $F_5$). The plot of cumulative percentage V/s time (min) for all formulations was plotted and depicted in figure (7) respectively.

## CONCLUSION

Various formulations ($F_1$-$F_5$) were developed by using suitable polymer (Carbopol 941). Topical liposomal gels were prepared by incorporation of liposome dispersion into structured vehicle carbopol (1%). Alternatively hydrogels containing acyclovir were prepared and their drug release properties were investigated. Drug-excipients compatibility study by FTIR showed no interaction between drug and selected excipients. Entrapment efficiency % was optimized after studying the effect of various ratios and formulation process. Maximum entrapment efficiency was 78.3 % with formulation $F_3$.Maximum drug content from optimized bath i.e. $F_3$ was found to be 91.33%.The percentage cumulative drug release from optimized bath i.e. $F_3$ was found to be 101.25 after four hour of diffusion studies. So it is suitable for topical application.

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