



## Formulation and Evaluation of Acyclovir Liposomes

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### Article info

Article history:  
Received 12 JAN 2017  
Accepted 04 FEB 2017

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### Abstract

Acyclovir is a synthetic acyclic purine nucleoside analog that is currently used for the prevention and treatment of Herpes Simplex Virus (HSV) and Varicella-Zoster Virus (VZV) infection that occurs at epidermis. The oral bioavailability of acyclovir is low, variable and species dependent. The aim of the study is to prepare 10 formulations of liposomal carrier for Acyclovir for the treatment of viral infections that is capable of delivering the drug to the specific target site by topical route by using different ratios of phospholipids and cholesterol with a desired amount of drug by thin film hydration technique and rotary flash evaporation technique and to find out the drug release from the liposome's of different ratios, drug release pattern and also to find out the size distribution of liposome's of different ratios and also to increase the bioavailability and efficacy of the drug.

**Keywords:** Herpes Simplex Virus (HSV), Varicella-Zoster Virus (VZV), acyclovir

### INTRODUCTION

Liposomes are microscopic structures consisting of one or more concentric spheres of lipid bilayer, enclosing aqueous compartments [1]. As drug carrier systems, liposomes have been noted to be superior over conventional preparations. Phospholipids, being the major component of liposomal systems, can easily get integrated with the skin lipids and maintain the desired hydration conditions to improve drug penetration and localization in the skin layers [2-4]. Thus, recognizing the need for topical delivery of Acyclovir and the promising potential of liposomes, it has been envisaged to entrap the drug into these carriers. Interestingly, the incorporation of Acyclovir into lipid bilayers has been viewed to bring additional benefit of

imparting stability to the liposomes. The latter is related to the cholesterol like structure of Acyclovir which on its incorporation in liposomes reduces the flux of molecules. Acyclovir is a synthetic acyclic purine nucleoside analog that is currently used for the prevention and treatment of Herpes Simplex Virus (HSV) and Varicella-Zoster Virus (VZV) infection that occurs at epidermis [5]. The oral bioavailability of acyclovir is low, variable and species dependent. The pharmacokinetic properties of acyclovir are well established. The effects of dosage size on the extent of oral absorption are not well understood. Some reports suggest from the gastrointestinal tract may be a saturable, dose dependent process [6]. Acyclovir is also categorized class III drug according to the Biopharmaceutical Classification System (BCS) because of its high solubility and low permeability [7]. The current study includes the preparation of Acyclovir-loaded liposomes by investigating the Influence of different formulation and process related variables. Various parameters viz. percent drug loading (PDL), vesicular size distribution and drug-leakage profile has been assessed. Comparative evaluation of developed liposomes was conducted for their geometric mean diameter, percent drug entrapment and for *in vitro* drug diffusion studies across gelatin sheet. Results of diffusion study of both types of liposomes were related to determine the lamellarity of the liposomes considering total deposition of liposomal acyclovir in layers of gelatin sheet prepared by TFH and RFE methods.

## **MATERIALS AND METHODS**

Acyclovir was a kind gift from Mylan laboratories. India. Soya Lecithin (PC) (solid) and Cholesterol (CHOL) are purchased from SD Fine Chemicals, Mumbai. All the excipients and reagents were used as received of AR Grade. Distilled water was prepared freshly whenever required.

### **Preformulation study of Acyclovir**

#### **Solubility analysis**

Excess amount of drug was taken in a 5 ml of the solvent system. Stir it for 30 minutes. Keep it on a shaker for 48 hrs to achieve equilibrium and centrifuge it for 10 minutes, the supernatant layer is then filtered out. The dilutions are made from 10-50 µg /ml and the solubility is determined by measuring the UV absorption.

#### **Determination of absorption maxima ( $\lambda$ max) for acyclovir**

2 ml of stock solution (100 µg/ml) was taken in 10 ml volumetric flask and volume was made up to 10 ml with solutions of pH 7.4 PBS (20 µg/ ml) and scanned on a double beam spectrophotometer against respective media blanks. An absorption maximum ( $\lambda$  max) of 255.5 nm was obtained. This  $\lambda$  max was selected for preparation of standard curve of acyclovir in different media.

### FT-IR study

Dry sample of drug and potassium bromide was mixed uniformly and filled into the die cavity of sample holder and an IR spectrum was recorded using diffuse reflectance FTIR spectrophotometer (460 plus, Jasco).

### Preparation of acyclovir liposomes

#### Rotary flash evaporation method

Acyclovir multilamellar liposomal vesicles were prepared by using rotary flash evaporation technique. Five formulations were prepared by lecithin as lipid component with cholesterol. Accurately weighed quantities of drug, lecithin with cholesterol were transferred to 250ml round bottom flask and dissolved in diethyl ether (3ml). A layer of lipid film was formed by evaporating the solvent system under reduced pressure using rotary evaporator. During this process, the conditions of the instrument such as temperature ( $55\pm 2^\circ\text{C}$ ) and speed (150rpm) were kept constant.

#### Thin film hydration method

Acyclovir multilamellar liposomal vesicles were prepared by using thin film hydration technique. Five formulations were prepared by lecithin as lipid component with cholesterol. Accurately weighed quantities of drug, lecithin with cholesterol were transferred to 250ml round bottom flask and dissolved in dichloromethane (3ml). A layer of lipid film was formed by evaporating the solvent system under reduced pressure using rotary evaporator. During this process, the conditions of the instrument such as temperature ( $40^\circ\text{C}$ ) and speed (100rpm) were kept constant.

**Table 1: Formulation of liposomes by rotary flash method**

Formulation Code	Cholesterol (mg)	Lecithin (mg)
RB1	100	100
RB2	100	200
RB3	100	300
RB4	100	400
RB5	100	500

**Table 2: Formulation of liposomes by thin film hydration method**

Formulation Code	Cholesterol (mg)	Lecithin (mg)
TB1	100	100
TB2	100	200
TB3	100	300
TB4	100	400
TB5	100	500

### Characterization of liposomes

#### Drug entrapment efficiency (or) drug content

Drug entrapped within the liposomes was estimated after removing the unentrapped drug, which was separated by collecting the supernatant after subjecting the dispersion to centrifugation in a cooling centrifuge at 1000 rpm at a temperature of 4°C for 30 minutes, where upon the pellets of liposomes were washed again with PBS to remove unentrapped drug. Then it was analyzed at 255 nm.

#### Percentage of entrapment efficiency

It is determined by using the ratio of entrapped drug to the total drug.

% Drug entrapped (PDE) = (amount of drug in sediment/ total amount of drug) × 100

#### *In vitro* diffusion studies

The *in vitro* diffusions studies were carried out by using 500 ml beaker containing 250 ml phosphate buffer pH 7.4. The beaker was assembled on a magnetic stirrer and the medium was equilibrated at 37 ± 5°C. dialysis membrane was taken and one end of the membrane was sealed. After separation of non-entrapped acyclovir liposome dispersion was filled in the dialysis membrane and other end was closed. The dialysis membrane containing the sample was suspended in the medium. The assay process was done by withdrawing 1 ml of sample at regular intervals for 12 hours and immediately replaced by the same quantity of buffer in to the donor component. The received samples were assayed by UV spectrophotometer at 255 nm.

#### Particle size analysis

The particle size of liposomes was determined by using scanning electron microscope. Optimized batch of liposomes were viewed under microscope to study their size. Size of liposomal vesicles was measured at different locations on slide by taking a small drop of liposomal dispersion on it and average size of liposomal vesicles was determined.

## Zeta potential

This method is used to determine charge on empty and drug loaded vesicles surface using Zeta sizer 300HSA (Malvern instruments, Malvern UK). Analysis time was kept for 60 seconds and average zeta potential and charge on the liposome was determined. The obtained value indicates that the surface of liposomes is dominated by the anions and proved that liposome have sufficient charge to avoid aggregation of vesicles.

## RESULTS AND DISCUSSION

### Solubility analysis

Table 3: Solubility profile of Acyclovir

S. No.	Solvent	Solubility
1	Distilled water	Slightly soluble
2	Ethanol	Very slightly soluble
3	Dilute hydrochloric acid.	Soluble
4	Dimethyl sulfoxide	Freely soluble

### Determination of absorption maxima ( $\lambda_{max}$ ) for acyclovir

2 ml of stock solution was taken in 10 ml volumetric flask and volume was made up to 10 ml with solutions of pH 7.4 PBS (20 ug/ ml) and scanned on a double beam spectrophotometer against respective media blanks. An absorption maximum ( $\lambda_{max}$ ) of 255.5 nm was obtained. This  $\lambda_{max}$  was selected for preparation of standard curve of acyclovir in different media. The graph is shown in fig.1.

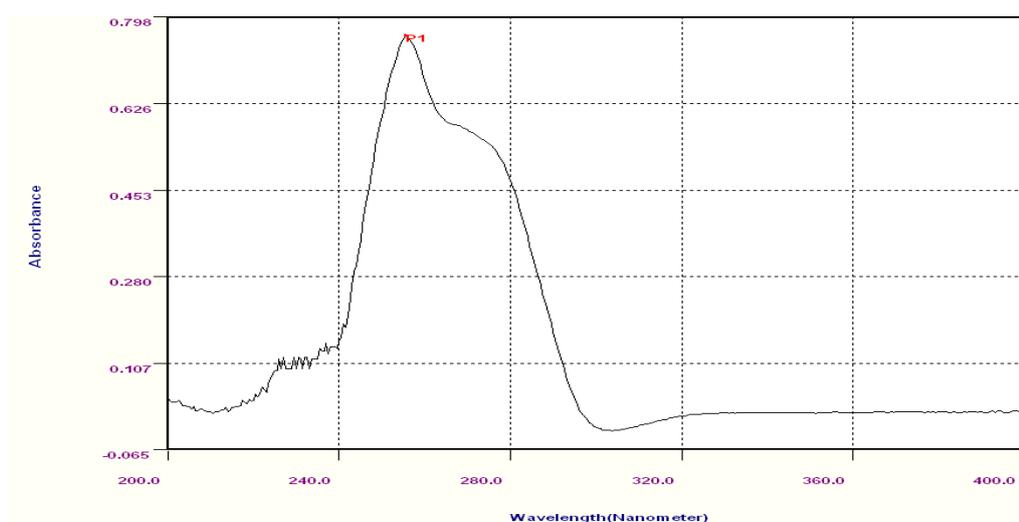


Figure 1:-  $\lambda_{max}$  for acyclovir

### Preparation of standard curve for acyclovir [Standard curve in PBS (pH 7.4)]

From the stock solution (100 µg/ml) aliquots of 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 4.5, and 5 ml solution were taken and diluted to 10 ml to obtain concentrations from 5 to 50µg/ml with PBS. The absorbance of solutions was determined at  $\lambda$  max 255.5 nm against PBS as blank. The experiment was repeated three times and calibration curve was plotted from the mean value. The result was shown in the fig.2.

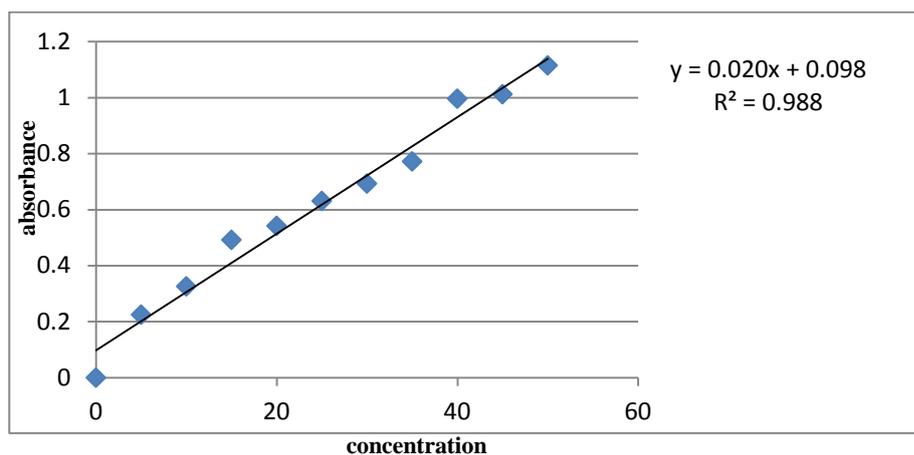


Figure 2: Standard curve of Acyclovir in PBS

### Standard curve in distilled water

From the stock solution B- 100 µg/ml, aliquots of 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 4.5, and 5 ml solution were taken and diluted to 10 ml to obtain concentrations from 5 to 50µg/ml with distilled water. The absorbance of solutions was determined at  $\lambda$  max 255.5 nm against distilled water as blank. The experiment was repeated three times and calibration curve was plotted from the mean value. The result was shown in fig.3

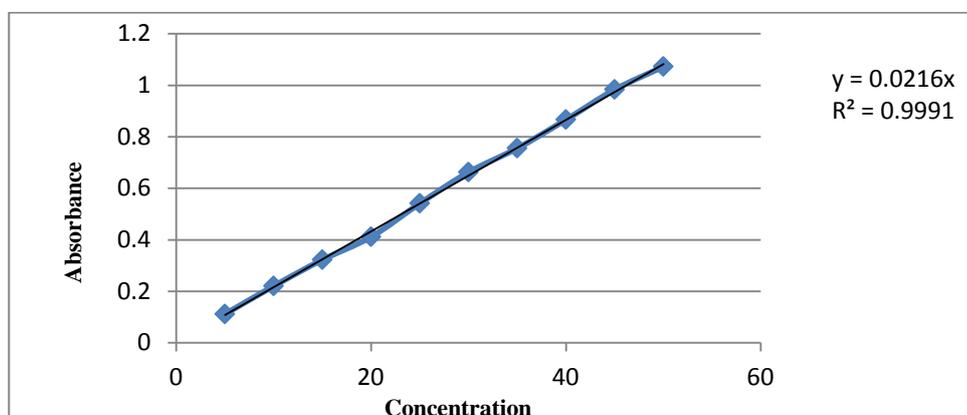


Figure 3:-Standard curve of Acyclovir in distilled water

### FTIR Study

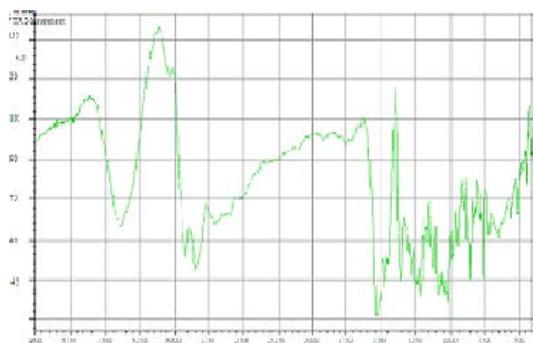


Figure 4: IR Spectra of Acyclovir

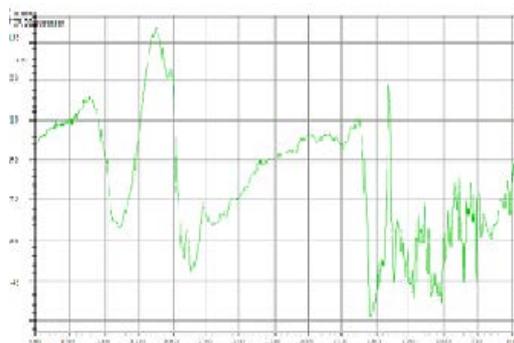


Figure 5: IR Spectra of physical mixture of drug and polymer

No significant change in peak pattern in the IR spectra of pure Drug and combination of drug with polymer indicates no interaction between pure drug and polymer.

### Characterization of liposomes

#### Drug entrapment efficiency (or) drug content and entrapment efficiency

Table 4: Drug entrapment and % entrapment efficiency of the formulations

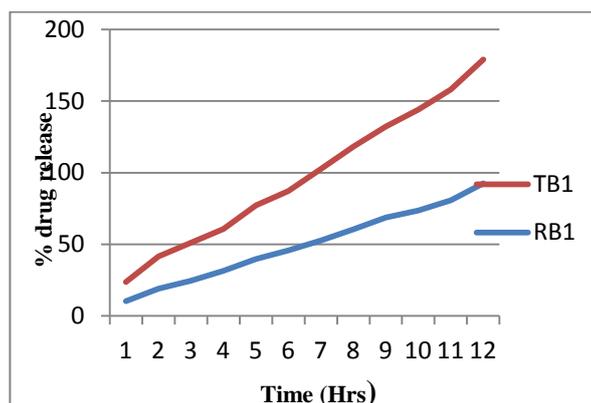
S. No	Formulation Batches	Drug entrapment	% entrapment efficiency
1	RB1	9.6±0.04	96%
2	RB2	9.4±0.05	94%
3	RB3	9.2±0.04	92%
4	RB4	9.5±0.03	95%
5	RB5	9.1±0.01	91%
6	TB1	9.9±0.03	99%
7	TB2	9.8±0.01	98%
8	TB3	9.6±0.05	96%
9	TB4	9.7±0.04	97%
10	TB5	9.8±0.02	98%

Drug entrapped within the liposomes was estimated after removing the untrapped drug, which was separated by collecting the supernatant after subjecting the dispersion to centrifugation in a cooling centrifuge at 1000 rpm at a temperature of 4°C for 30 minutes,

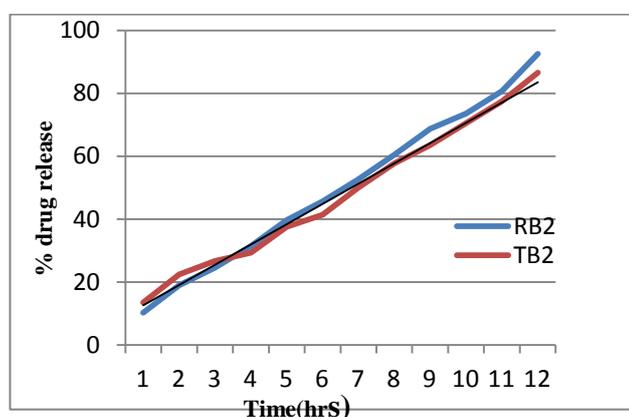
where up on the pellets of liposomes were washed again with PBS to remove untrapped drug, Then analyzed at 255 nm. Results shown in Table No 4.

### ***In vitro* diffusion studies**

The *in vitro* diffusions studies were carried out by using 500 ml beaker containing 250 ml phosphate buffer pH 7.4. The beaker was assembled on a magnetic stirrer and the medium was equilibrated at  $37 \pm 5^{\circ}\text{C}$ . dialysis membrane was taken and one end of the membrane was sealed. After separation of non-entrapped acyclovir liposome dispersion was filled in the dialysis membrane and other end was closed. T



**Figure 6: percentage drug release in RB1 & TB1**



**Figure 7: percentage drug release percentage drug release in RB2 & TB2**

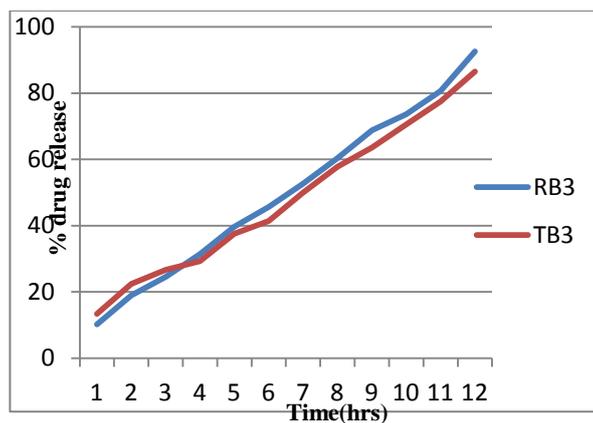


Figure 8: percentage drug release in RB3 & TB3

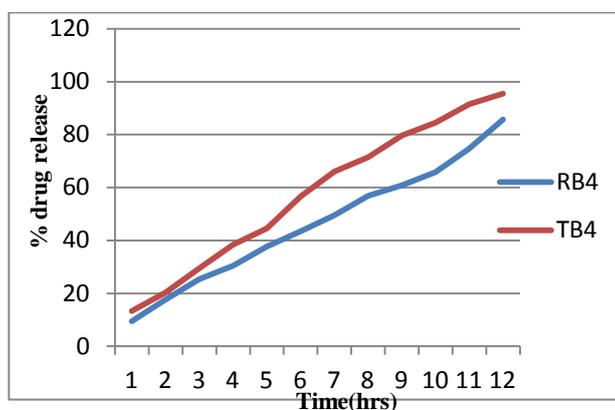


Figure 9: percentage drug release in RB4 & TB4

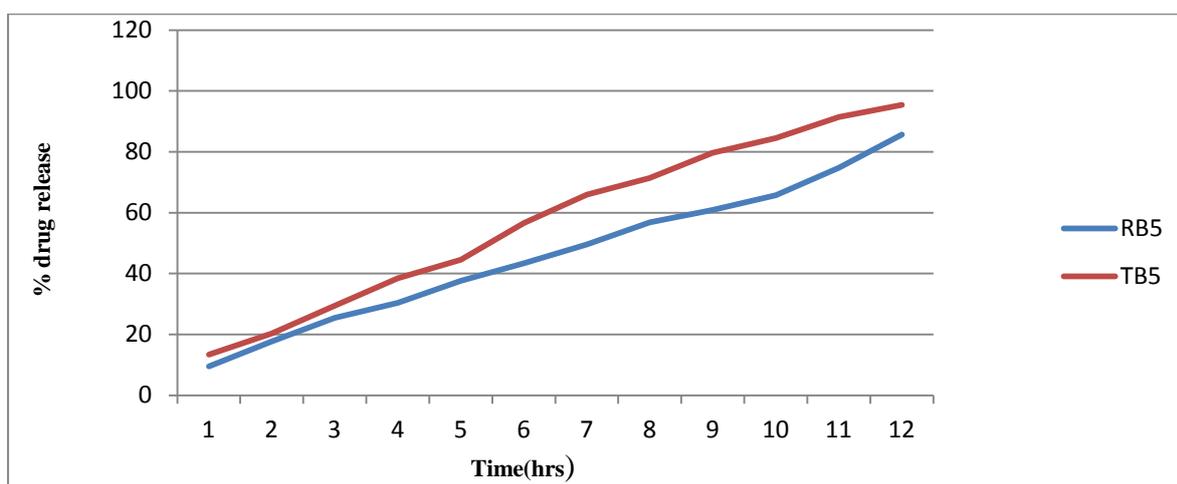


Figure 10: percentage drug releases in RB5 & TB5

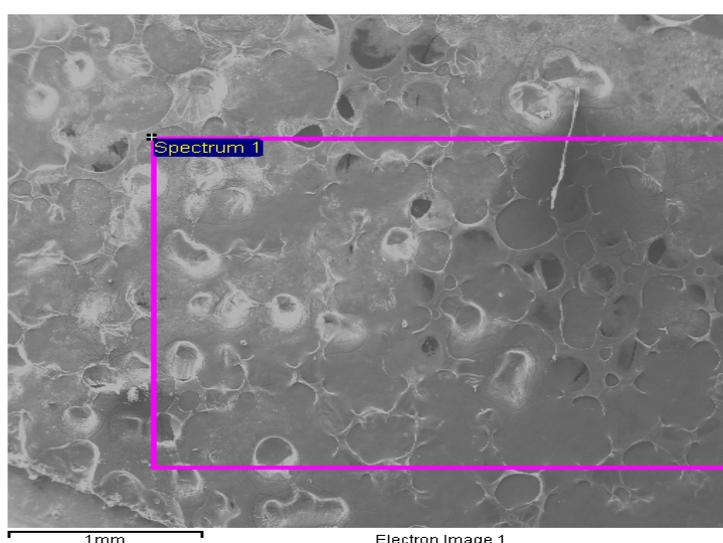
The dialysis membrane containing the sample was suspended in the medium. The assay process was done by withdrawing 1 ml of sample at regular intervals for 12 hours and

immediately replaced by the same quantity of buffer in to the donor component. The received samples were assayed by UV spectrophotometer at 255 nm.

Formulations RB1 and TB1 containing cholesterol and lecithin in the ratios of 1:1 had % cumulative release of 98.80% and 99.83% in 12 hrs respectively. The release profile is depicted in figure 6, when the concentration increases which shows that cholesterol and lecithin was helpful in retarding the drug release. Formulations RB2 and TB2 containing cholesterol and lecithin in the ratios of 1:2 had % cumulative release of 98.90% and 99.75% in 12 hrs respectively. The release profile was shown in the figure 7, which shows that the drug was released in a controlled manner. Formulations RB3 and TB3 containing cholesterol and lecithin in the ratios of 1:3 had % cumulative drug release of 83.98% and 93.78% in 12hrs respectively. The release profile was shown in figure 8. Formulations RB4 and TB4 containing cholesterol and lecithin in the ratios of 1:4 had % cumulative drug release of 92.56% and 86.54% in 12hrs respectively. The release profile was shown in figure 9. Formulations RB5 and TB5 containing cholesterol and lecithin in the ratios of 1:5 had % cumulative drug release of 85.66% and 95.43% in 12hrs respectively. The release profile was shown in figure 10. It was also observed that cumulative drug release increased when the concentration of the cholesterol and lecithin was increased. Formulation TB1 contains cholesterol and lecithin (1:1) which has shown highest % cumulative drug release upto 99.83%.

### **Particle size analysis**

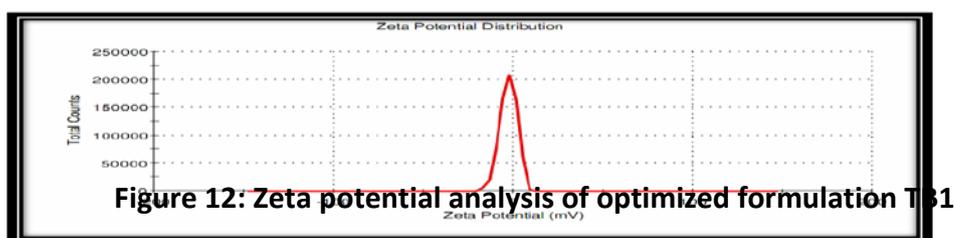
The particle size of liposomes was determined by using scanning electron microscope. Optimized batch of liposomes were viewed under microscope to study their size. Size of liposomal vesicles was measured at different locations on slide by taking a small drop of liposomal dispersion on it and average size of liposomal vesicles was determined.



**Figure 11: particle size analysis of optimized formulation of TB1**

## Zeta potential

This method is used to determine charge on empty and drug loaded vesicles surface using Zeta sizer 300HSA (Malvern Instruments, Malvern, UK). Analysis time was kept for 60 seconds and average zeta potential and charge on the liposome was determined. The zeta potential of optimized formulation (TB1) which is selected based on Poly Dispersity Index is shown in Figure 23. The value was -20 mv which indicates that the surface of liposomes is dominated by the anions and proved that prepared liposome have sufficient charge to avoid aggregation of vesicles.



## CONCLUSION

The different formulations of liposomes containing Acyclovir were prepared by using Rotary flash evaporation and thin film hydration method. The percentage entrapment efficiency was determined and maximum entrapment efficiency was found to be 99%. The optimized batches are found to have good entrapment efficiency and it was proved that as the concentration of the cholesterol increased the particle size also increased and entrapment efficiency decreased. In formulation TB1 the ratio of cholesterol and lecithin is 1:1 is selected as optimized formulation based on entrapment efficient and diffusion studies Optimized formulation was evaluated for particle size analysis and zeta potential. From the above study, we concluded that Acyclovir can be formulated as liposomes with phospholipids such as lecithin and cholesterol. FTIR studies were done on the pure drug and physical mixture of drug and polymers. From FTIR spectra of the drug and physical mixture it was found that there is no significant interaction. Zeta potential analysis was done for optimized formulation TB1. Average zeta potential and charge on the liposome was determined. The value was -20 Mv which indicates that the surface of liposomes is dominated by the anions and proved that prepared liposome have sufficient charge to avoid aggregation of vesicles.

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