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DEVELOPMENT AND CHARACTERIZATION OF ACYCLOVIR LOADED LIPOSOME USING ROTARY FLASH EVAPORATION TECHNIQUE

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ABSTRACT

The objective of the present study is to form liposomal based acyclovir ointment. Acyclovir, an antiviral drug is widely used in the treatment of herpes simplex (types 1 and 2). Acyclovir is a synthetic purine nucleoside analogue with *in vitro* and *in vivo* inhibitor activity against herpes simplex virus types 1(HSV-1), 2 (HSV-2) and varicella-zoster virus (VZV) that occurs at epidermis. The oral bioavailability of acyclovir is low. Five formulations of liposomal carrier for Acyclovir prepared 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 rotary flash evaporation technique. and to find out the drug entrapment 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 (ACV).

INTRODUCTION

Liposomes are microscopic tiny bubbles like structures consisting of one or more concentric spheres of lipid bilayer, enclosing aqueous compartments[1]. Liposomes can be filled with drugs, and used to conveyvaccines, drugs, enzymes or other substances to target cells organs and drugs for cancer and other diseases. As drug carrier systems, liposomes have been noted to be superior over conventional preparations. Phospholipids are may be 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[2,3,4]. 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. 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 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].

MATERIALS AND METHODS OF LIPOSOMAL ACYCLOVIR

Acyclovir was a kind gift from Cadila Healthcare Ltd. Ahmedabad, India. Soya Lecithin (PC) (solid) and Cholesterol (CHOL) are used of a manufacturer HiMedia Laboratories, SPAN 60 from CENTRAL DRUG HOUSE (P) ltd. and potassium dihydrogen phosphate from MERCK Specialities pvt ltd. which is provided by TIT pharmacy, Bhopal. All the excipients and reagents were used as received of AR Grade.

PREFORMULATION STUDY:

Preformulation constitutes an important step in the layout Plan for the design of drug delivery systems. The motive behind Preformulation study was to prove physicochemical parameters of drug, physical characteristics and compatibility with common excipients. Preformulation study was mainly performed to check the purity of drug and interaction with excipient. Various parameters like melting point, pKa, Ko/w, flow properties of the powder drug, crystal morphology, drug excipients compatibility studies and particle size distribution were performed. The overall purpose of preformulation testing is to generate information useful to developing stable and safe dosage forms with good bioavailability. There are many steps can be used in preformulation process like Physicochemical properties, melting point, particle size shape, Infrared analysis, Thin-layer chromatography, Organoleptic properties, pKa determinations, Partition coefficient, pH- solubility profile, Prodrug approach, Polymorphism and Solubility[8].

Organoleptic properties of acyclovir

Appearance, colour, odour studies were performed by visual assessment without using any special type of equipment.

Solubility

It was determined by taking 10 mg of drug sample in successively increasing amount in water, ether, alcohol and chloroform.

Hygroscopicity

Samples are accurately weighed in a tarred container and placed at various humidity conditions for periods up to two weeks. Weight gain or loss is measured at predetermined time intervals until equilibrium is reached. If the drug is very hygroscopic or unstable in the presence of moisture, the drug would have to be stored under dry conditions.

High hygroscopicity is not good for many reasons, including Handling problems, requirement of special storage conditions, chemical and physical stability problems.

Melting Point

Melting point of acyclovir was determined by using open capillary tube method. Pure drug was placed in a capillary tube which was fused at one end and placed in a digital melting point apparatus. The temperature at which drug begin start melting was noted.

Partition Coefficient

It is defined as the ratio of unionized drug distributed between the organic and aqueous phases at equilibrium.

Shake flask method is used to determine partition coefficient. Partition coefficient of acyclovir determined by taking 5ml of octanol and 5ml of methanol and about 10mg of acyclovir added in to this solution and was shaken and stand it for 24 hrs. two layer were separate by separating funnel and filter through WHATMAN grade filter. The amount of acyclovir solubilised, was determine by measuring the absorbance at 254nm against reagent blank through double beam UV/vis spectrophotometer in both the solution partition coefficient was determined as ratio of concentration of drug in octanol to the concentration in phosphate buffer (pH 7.4) and value were reported in logP[10].

$$\mathbf{K_{ow}} = \frac{\text{concentration of drug in non aqueous phase}}{\text{Concentration of drug in aqueous phase}}$$

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DSC of pure drug

DSC of acyclovir drug was performed by PerkinElmer Thermal Analysis with 3.200 mg of sample by holding for 1.0 min at 30.00°C and heat from 30.00°C to 300.00°C at 20.00°C/min.

DSC of mixture

Differential scanning calorimetry was done for physical mixture of drug and polymers (acyclovir, cetostryl alcohol, soya lecithin, cholesterol, span60). DSC of mixture is calculated by PerkinElmer Thermal Analysis by holding 3.220 mg of sample for 1.0 min at 30.00°C and heat from 30.00°C to 300.00°C at 20.00°C/min. Data reported in figure (3).

Infrared Spectroscopy

Infrared spectrum of any compound or drug gives information about the group present in that particular compound. FTIR studies were done on the pure drug (acyclovir) by Shimadzu instrument, Japan. From FTIR spectra of the drug it was found that this is pure drug which used for formulation.

PREPRATION OF LIPOSOME BASED ACYCLOVIR

Rotary flash evaporation method

Liposome was prepared according to the method reported by Avinash and Ambikanandan. Acyclovir liposomal vesicles were prepared by using rotary flash evaporation technique[9]. Five formulations were prepared by lecithin as lipid component with cholesterol. Accurately weighed quantities of drug, lecithin(1mg) with cholesterol(1mg) and span60 1ml 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 (50±52ºC) and speed (150rpm) were kept constant. Then this film is hydrated by phosphate buffer.

Table no.1: Formulation of Liposomal based acyclovir

Formulation code	Drug	Lecithin	Cholesterol	Cetosteryl alcohol	Span 60
F1	50mg	100mg	100mg	100mg	1ml
F2	50mg	200mg	100mg	100mg	1ml
F3	50mg	300mg	100mg	100mg	1ml
F4	50mg	400mg	100mg	100mg	1ml
F5	50mg	500mg	100mg	100mg	1ml

Preparation of Ointment

Semisolid acyclovir liposome was prepared by adding cetosteryl alcohol. 1mg Cetosteryl alcohol add in to ml of liquid paraffin slightly heat for mixing then add 2ml of liposomal acyclovir in this mixture with continuously stirring a semisolid preparation were formed.

CHARACTERIZATION OF FORMULATION

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 40^0C 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.

Phase Response and transitional behaviour

Liposome and lipid bilayer exhibit various phase transition that are studied for their role in triggered drug release or stimulus mediated fusion of liposomal constituent with target cell. An understanding of phase transition and fluidity of phospholipids membrane is important both in manufacture and exploitation of liposomes since phase behaviour of liposomal membrane determine such properties such as permeability, fusion, aggregation and protein binding. The phase transition has been evaluated using freeze fracture electron microscopy. They are more comprehensive verified by differential scanning calorimeter analysis.

DSC of formulation

Differential scanning calorimetric (DSC) curve of liposome based acyclovir formulation is determined by PerkinElmer Thermal Analysis by taking 4.550 mg sample) by holding for 1.0 min at 30.00°C and heat from 30.00°C to 300.00°C at 20.00°C/min.

Zeta sizer

Particle size of liposome determine by two zeta sizer method (1) size distribution by volume (2) size distribution by intensity. Particle size of formulations were determined by Zetasizer DTS (Malvern Instrument, UK)

***In vitro* drug release study of liposome formulation**

The *in vitro* drug release study of acyclovir containing liposome formulation was performed with diffusion cell at 30±2°C temperature. Receptor compartment of diffusion cell contained 50 ml 25% v/v PEG 400 in phosphate buffer (pH-7.4) solution and was constantly stirred by a magnetic stirrer (Expo India Ltd., Mumbai, India) at 60 rpm. Dialysis membrane (molecular weight cut off 12 KD, HiMedia Laboratories Pvt. Ltd., India) was employed as release barrier in between receptor and donor compartment which was previously was with distilled water and soaked with 25% v/v PEG 400 solution. Sample of 5 ml was withdrawn through the sampling port of the diffusion cell in intervals one h, over 7 h and same volume of 25% v/v PEG 400 solution was replaced immediately. The samples of *in vitro* release study were suitably diluted and analyzed by HPLC (Shimadzu, Japan) at 254 nm for drug content[11, 12].

RESULT AND DISCUSSION

Solubility Study

Table 2: Solubility Analysis of Acyclovir

S.No	Solvent	Solubility
1	Water	Sparingly soluble
2	PBS(pH7.4)	Sparingly soluble
3	0.1N NaOH	Freely soluble
4	DMSO	Freely soluble
5	0.1N HCL	Freely soluble

Melting point

Melting point of acyclovir was found to be 256.5-257.1^{0}c which is according to the standard melting point of the acyclovir. Standard melting point of the acyclovir is 256.5^{0}c(493.7^{0}F).

Partition Coefficient

Partition Coefficient was determined as ratio of concentration of drug in octanol to the concentration of drug in water and value were reported 0.026.

DSC of pure drug

DSC of acyclovir drug was performed by PerkinElmer Thermal Analysis with 3.200 mg of sample by holding for 1.0 min at 30.00°C and heat from 30.00°C to 300.00°C at 20.00°C/min. Data reported in figure (1).

DSC of mixture

Differential scanning calorimetry were done for physical mixture of drug and polymers (acyclovir, cetostryl alcohol, soya lecithin, cholesterol, span60). DSC of mixture is calculated by PerkinElmer Thermal Analysis by holding 3.220 mg of sample for 1.0 min at 30.00°C and heat from 30.00°C to 300.00°C at 20.00°C/min. Data reported in figure (2).

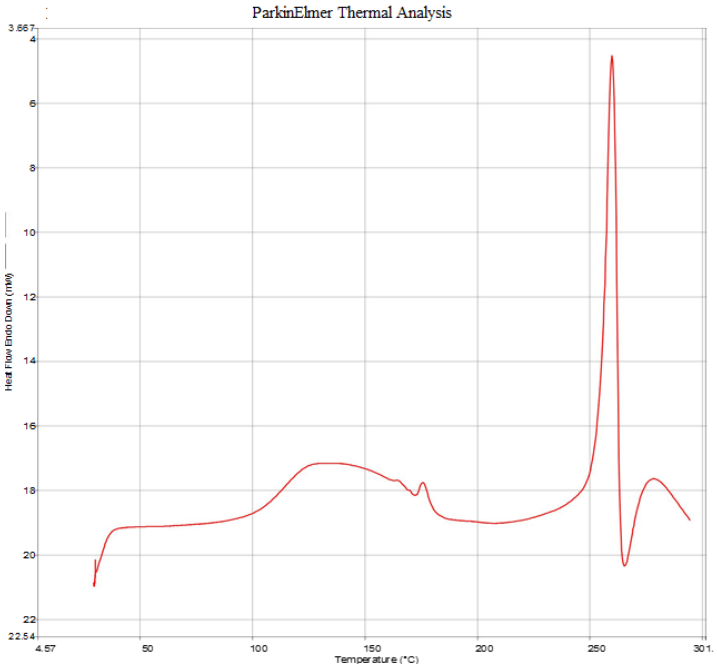


Figure 1: DSC of Acyclovir

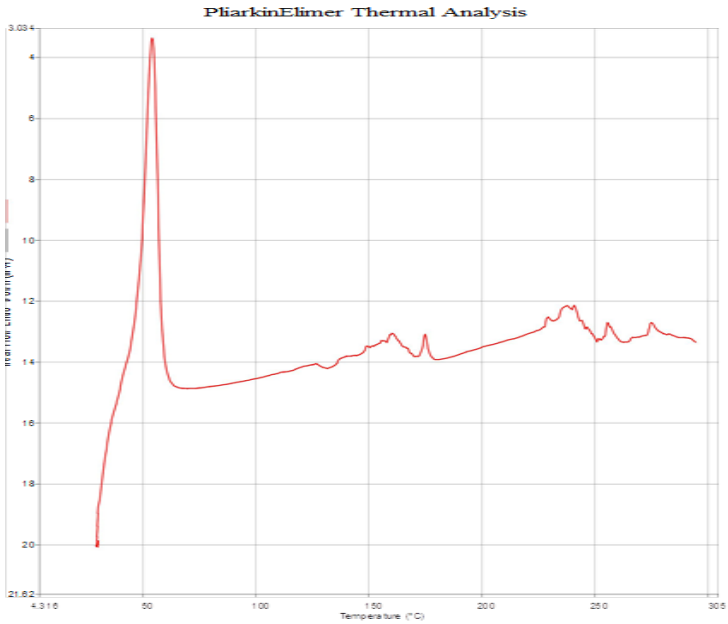


Figure 2: DSC of mixture

FTIR

FTIR studies were done on the pure drug (acyclovir) by Shimadzu instrument, Japan. From FTIR spectra of the drug it was found that this is pure drug which used for formulation. The report has shown in figure (3).

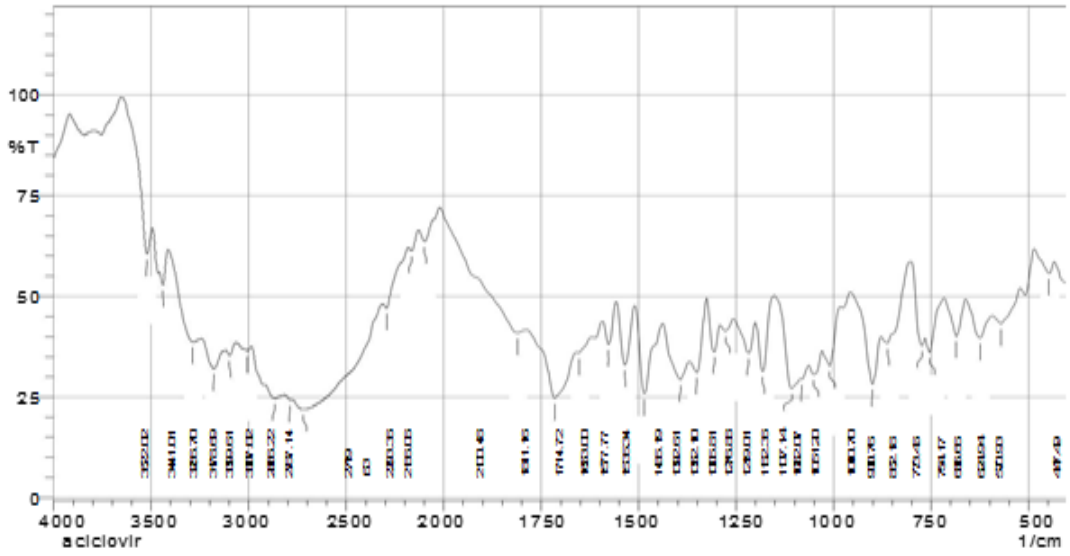


Figure 3: IR Spectra of Acyclovir

Charecterization of Liposomal Formulation:

Entrapment Efficiency of the formulation:

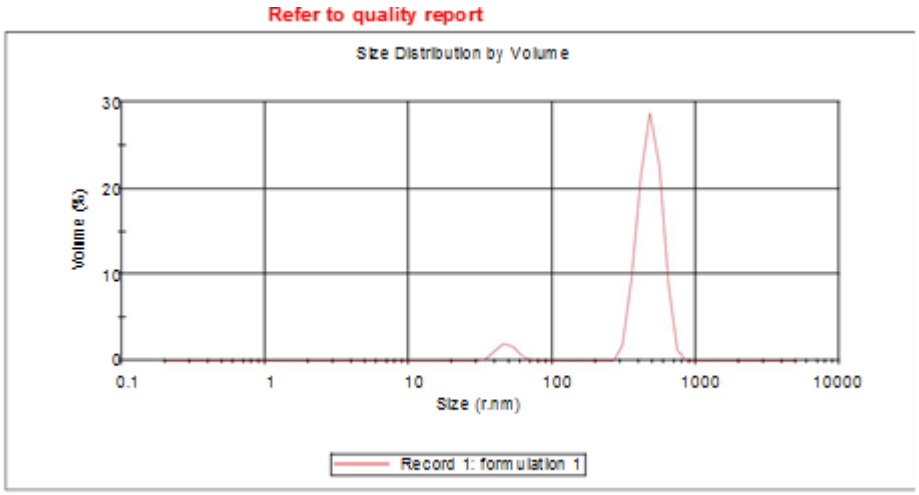
Table 3: Entrapment Efficiency of different formulation:

Formulation batch	Drug entrapment	%entrapment efficiency
F1	5.4±0.04	54%
F2	5.2±0.04	52%
F3	5.6±0.05	56%
F4	4.8±0.01	48%
F5	4.7±0.06	47%

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 40^0C 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. The percentage entrapment efficiency was determined in five batches of formulation in which maximum entrapment efficiency was found in F3 which was 56%.

Zeta sizer

Particle size of liposome determine by two zeta sizer method: (1) size distribution by volume (2) size distribution by intensity. Particle size of formulations were determined by Zetasizer DTS (Malvern Instrument, UK) data reported in figure (4). Particle size of liposome reported 484.4r. nm in major width (88.30r.nm) and 48.16r.nm in minor width (7.611r.nm).



Results

		Size (r.nm):	% Volume	Width (r.nm):
Z-Average (r.nm): 1604	Peak 1:	484.4	94.2	88.30
PdI: 0.701	Peak 2:	48.16	5.8	7.611
Intercept: 0.859	Peak 3:	0.000	0.0	0.000

Result quality :

Figure.4: zeta sizer of liposome based acyclovir optimized formulation (size distributon by volume)

***In vitro* drug release study of liposome formulation**

Maximum drug release of formulation F1 reported 76.9% in 7h. Liposome shows steady release of drug in controle manner.

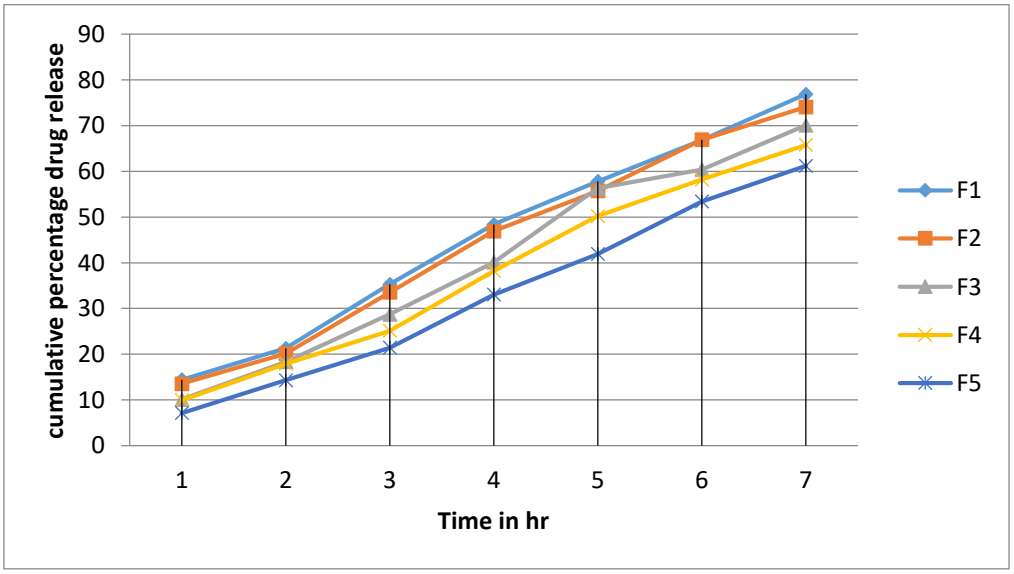


Figure.5: *In vitro* release study of liposome formulations

CONCLUSION

The different formulations of liposomes containing Acyclovir were prepared Rotary flash evaporation method. The percentage entrapment efficiency was determined and maximum entrapment efficiency was found to be 56%.

Differential scanning calorimetry were done for pure drug (acyclovir), physical mixture of drug and polymers (acyclovir, cetostryl alcohol, soya lecithin, cholesterol, span60) and semisolid formulation of liposome based acyclovir. It is use to observe crystallization temperature, glass transition temperatures, oxidation, as well as other chemical reactions.

FTIR studies were done on the pure drug (acyclovir). From FTIR spectra of the drug it was found that this is pure drug which used for formulation. Zeta sizer analysis was done for optimized formulation (liposome based acyclovir). Average zeta size of liposome was determined. Particle size of liposome reported 484.4r.nm in major width (88.30r.nm) and 48.16r.nm in minor width (7.611r.nm).

In this study acyclovir liposomal topical ointment design has to be prepared, using combination of phosphatidyl choline and cholesterol. So that drug can release in a control rate from the liposomal preparation to the skin. Various combinations of the phosphatidyl choline and cholesterol are used in different proportions for the preparation of liposome of different batch, and their drug release profile is seen, to optimize the process to find the best formulation that shows good drug release pattern. Stability, zeta potential, encapsulation efficiency is determined.

In summery in this research we have made progress towards establishing a method for producing and testing of liposomes as well as identifying a formulation that effectively can incorporate the drug in circulation. However, we have yet to arrive at the ideal formulation, and, as we are still evaluating elements that affect all areas of incorporation and retention, further studies are needed in following order to reach the goal of a formulation appropriate for *in vivo* studies.

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