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Original Article

DESIGN AND *IN VITRO* RELEASE KINETICS OF LIPOSOMAL FORMULATION OF ACYCLOVIR

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ABSTRACT

Objective: The objective of this study was to formulate and evaluate liposomes loaded with acyclovir.

Methods: Liposomal formulation of acyclovir was prepared by "thin lipid film method". Mixture of phosphotidyl choline (soya lecithin), acyclovir and cholesterol of varying weight ratio was used for the preparation of liposomes. Total nine batches were prepared and each batch was prepared in triplicate. All batches were evaluated by drug entrapment and release kinetic studies. Stability study was carried out with F_4 batch.

Results: F_2 and F_9formulations showed better drug entrapment efficiency compared to the other batches. The drug entrapment efficiency of all batches was found within the range of 51.42 % to 79.0 %. F_4 shows highest release of about 79.0% in 4 h. F_4 formulation was found to follow significantly zero-order release kinetics. Short term stability study of formulation F_4 showed no significant change in release kinetics.

Conclusion: The F_4 batches showed promising results compared to other formulations. No changes were founded during the short-term stability study of F_4.

Keywords: Liposomes, Acyclovir, Phosphatidylcholine, Cholesterol, Release kinetics

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INTRODUCTION

Liposomes are composed of a lipid bilayer which used as drug delivery vehicles. The hydrophobic chains of the lipids form the bilayer and the polar head groups of the lipids are towards the extra vesicular solution and inner cavity. Liposomes have polar and non-polar groups on the same molecule. Due to hydrophilic and lipophilic interactions they can form self-organized and ordered structures in solvents [1]. Liposomes can give ligand targeted, long-acting and triggered release systems. Liposome preparation techniques may be broadly classified into two types–bulk method and film method. In bulk method phospholipids are transferred from an organic phase into an aqueous phase. In the film method lipid films are deposited on a substrate followed by hydration to get the liposomes. Liposomal preparations have also been classified according to mean size, polydispersity and lamellarity of liposomes [2]. The hydrophilic part of liposomes is mainly phosphoric acid bound to water-soluble molecules. On the other hand hydrophobic part of liposome contains two fatty acid chains with 10 to 24 carbon atoms and each chain contains 0 to 6 double bonds [3].

Most antiviral agents which have activity against the herpes group of viruses belong to a class of compounds known as nucleosides. Acyclovir or 9-(2-hydroxyethoxy methyl) guanine is one of those antiviral agents [4]. Oral absorption of acyclovir is about 20% [5, 6]. Serious infections must be treated with intravenous acyclovir. The drug distributes all over the body. Cerebrospinal fluid (CSF) concentration is less than half of plasma concentration. The drug is excreted unchanged through kidneys. Excretion can be by both glomerular filtration and tubular secretion. Acyclovir has a half-life of about 2.5 to 3 h that can increase in renal impairment. Most importantly acyclovir used in encephalitis caused by herpes simplex, skin infections with herpes simplex, including labial and genital herpes can be treated by topically applied acyclovir [7, 8]. Acyclovir has different routes of administration like topical, oral and intravenous but it is discovered that intravenous formulation has most profound antiviral effect in case of *in vivo* study [9]. Intramuscular or subcutaneous administration of liposomal formulation in suspension form can increase the therapeutic efficacy of the encapsulated drug. When liposomal formulation was administered intramuscularly, toxicity of therapeutic drugs was found to be reduced [9-11]. Liposomes can be used as delivery vehicles for chemotherapeutics [12]. Human Immunodeficiency Virus (HIV)-infected persons have more risks of herpes-zoster than non-HIV patients. In those cases acyclovir can reduce incidence in HIV-infected persons [13].

MATERIALS AND METHODS

Materials

Acyclovir, Phosphatidyl Choline (soya lecithin), and cholesterol were purchased from Yarrow Chem, Mumbai. Chloroform was purchased from Merck, India. All the other chemicals and reagents used were of analytical grade.

Preparation of liposomes

For the preparation of acyclovir liposomes "Thin Lipid Film Method" was employed. A mixture of phosphatidylcholine (soya lecithin), acyclovir and cholesterol of specific ratio were dissolved in 5 ml of chloroform [14-16] with sonication for 10-20 min. The organic solution was taken in a round bottom flask and rotated on a thermostatic water bath (30 °C-35 °C) to evaporate the organic solvent [17]. The drying process was continued for about 40 min. The mixture of lipid and drug was deposited as a thin film in the round-bottom flask after complete evaporation of chloroform [18]. Proper drying plays a very important role in the preparation of liposomes [19]. After drying, 5 ml phosphate buffer (pH 7.4) was added and sonication was applied until a milky white suspension was formed (temperature maintained at 35 °C). Liposomes were formed due to spontaneous hydration of lipids in aqueous media. The suspension was allowed to stand for overnight. Sonication for 1 hour was applied to reduce the particle size [20].

Entrapment study

Liposomal suspension, 1 ml was taken in a glass centrifuge tube, followed by centrifugation [21] at 3500 rpm for 40 min. The supernatant was completely removed by a micropipette followed by washing the pellet with 5 ml of phosphate buffer solution (pH 7.4) to remove any un-entrapped drug. Both of the solutions were pooled and the clear supernatant solution was diluted. The absorbance was measured in UV-spectrophotometer (ELICO SA165) at 253 nm against phosphate buffer (pH 7.4) as blank.

In vitro drug release study

The drug release study of acyclovir from different liposomal formulations was carried out by diffusion technique [22]. Liposomal acyclovir suspension, 5 ml was taken in a dialysis bag [Abron Visking tube BE-253-b] [23, 24]. Both ends of the bag were clipped. The bag was immersed in a medium of phosphate buffer (pH 7.4). The medium was maintained at 37± 0.2 °C with constant stirring by a magnetic stirrer. Aliquots of samples (2 ml) of the medium were withdrawn at every 15 min interval up to 4 h. The volume of the medium was made up with 2 ml pre-warmed phosphate buffer (pH 7.4) [22, 23] after each sampling. The concentration of the sample was determined by taking the absorbance in UV-spectrophotometer (Elico SA165) at 253 nm against phosphate buffer (pH 7.4) as blank.

Drug stability study

Freshly prepared liposomal formulation of acyclovir that gave a better-sustained release pattern was stored in Eppendrof's tube at 4 °C [25] for one month. At the end of 1 mo, samples were analyzed by drug release study.

RESULTS

Drug entrapment efficiency

The un-entrapped drug was extracted out from the liposomal suspension with the help of phosphate buffer (pH 7.4) and the amount of un-entrapped drug was determined. The amount of drug entrapped was determined by subtracting from the amount of drug taken initially. Entrapment efficiency was determined from the following equation [26]:

$$\text{Drug entrapment efficiency} = \frac{\text{Actual drug content in liposomal acyclovir}}{\text{Theoretical drug content in liposomal acyclovir}} \text{ X } 100$$

The Drug Entrapment Efficiency (%) values found with various cholesterol proportions (mol %) are shown in fig. 1.

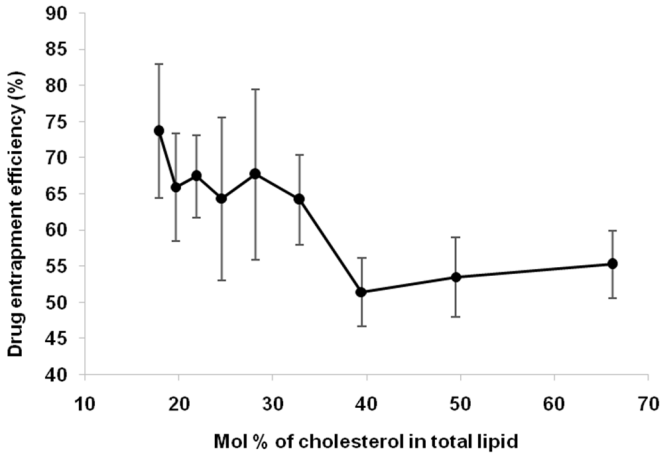

Fig. 1: Drug entrapment efficiency (%) versus mol % of the cholesterol in total lipid. (N=3, Mean ± SD)

From fig. 1 it is evident that % entrapment increases with a decrease in mol % of the cholesterol in the liposome and it became lowest at 39.5 mol% cholesterol (F_3), the lowest entrapment efficiency being 51.42%. The highest entrapment efficiency (73.6%) was found with F_9 having cholesterol of 17.9 mol%.

In vitro drug release study from liposomes

The dissolved acyclovir was diffused from liposome to the suspending medium and then permeated [27] across the dialysis membrane. Passage of the drug across dialysis membrane is a passive process. Therefore the rate of permeation was assumed to be higher than the rate of release of acyclovir from the liposome in the suspending medium. The percentage of drug released from the liposome was plotted against time and the release profiles of all the liposomal formulations are plotted in fig. 2. F_4 shows highest release of about 79.0% in 4 h.

Determination of t_{30} (Time required for 30 % drug release)

In order to compare between the release profiles of various formulations a parameter, time required for 30% release (t_{30}), was determined. The release rate was found to be highest in F_4 containing 32.9 mol% of cholesterol in the lipid phase which is exhibited by the lowest t_{30} value in fig. 3. The corresponding t_{30} value of F_4 was found to be 97.5 min.

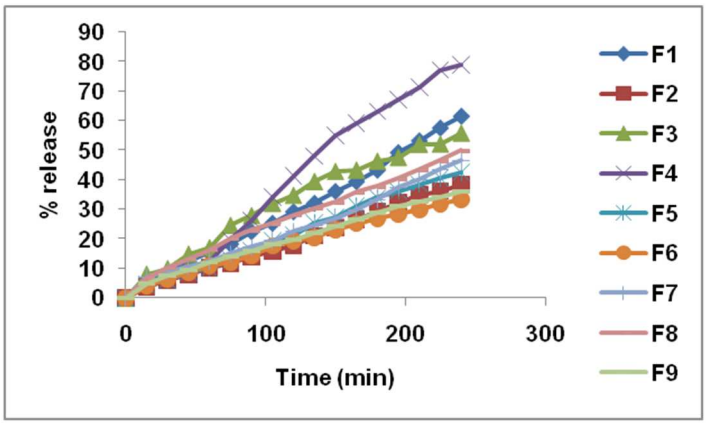

Fig. 2: Drug release study of F_1, F_2, F_3, F_4, F_5, F_6, F_7, F_8 and F_9

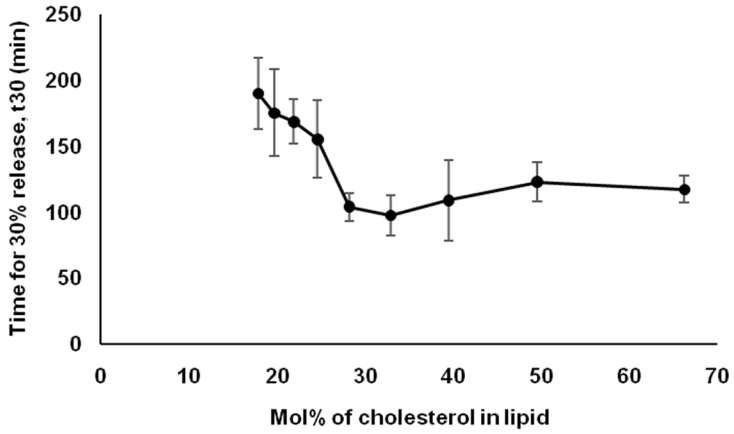

Fig. 3: Time taken for 30% release of drug from all formulations, (N=3, Mean ± SD)

***In vitro* drug release kinetic modeling**

The dissolved acyclovir was diffused from liposome to the suspending medium and then permeated [27] across the dialysis membrane. Passage of the drug across dialysis membrane is a passive process. Therefore the rate of permeation was assumed to be higher than the rate of release of acyclovir from the liposome in the suspending medium. In order to model the release kinetics of various liposomal formulations, the release profiles of drug from the liposomes were fitted to Zero-order, First-order, Higuchi, Hixon-Crowell, and Korsmeyer-Peppasequations. The correlation coefficients obtained from each model is displayed in table 1.

Table 1: Determination of coefficient of correlations of various kinetic models

Formulation	Zero-order	First-order	Higuchi	Hixon-crowell	Korsmeyer-peppas
F1	0.9923± 0.0088	0.9886± 0.0113	0.9176± 0.0482	0.9916 ±0.0066	0.8435 ±0.0520
F2	0.9893± 0.0164	0.9815 ±0.0196	0.9729 ±0.0052	0.9839 ±0.0187	0.8025 ±0.0499
F3	0.9624± 0.0417	0.9715± 0.0237	0.9711± 0.0061	0.9704± 0.0275	0.9026± 0.0565
F4	0.9810± 0.0171	0.9589 ±0.0142	0.9649 ±0.0066	0.9752 ±0.0076	0.7481 ±0.0390
F5	0.9954 ±0.0038	0.9888 ±0.0132	0.9735 ±0.0058	0.9730 ±0.0104	0.8564 ±0.0992
F6	0.9910 ±0.0056	0.9642 ±0.0547	0.9508 ±0.0426	0.9803 ±0.0254	0.7970 ±0.1374
F7	0.9826± 0.0214	0.9637± 0.0432	0.9761± 0.0128	0.9712± 0.0347	0.1474± 0.1522
F8	0.9923± 0.0088	0.9886± 0.0113	0.9176± 0.0482	0.9916± 0.0066	0.8435± 0.0520
F9	0.9970 ±0.0011	0.9965 ±0.0017	0.9733 ±0.0040	0.9546 ±0.0739	0.8581 ±0.0106

The mean values from 27 formulations (9 formulations x triplicate) are plotted against various kinetics models for comparison (vide fig. 4). From fig. 4 it is evident that the highest R^2 value was found with Zero-order release kinetics.

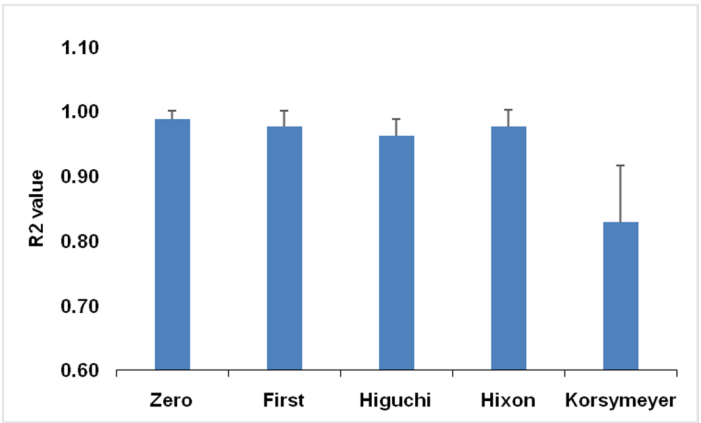

Fig. 4: The correlation coefficient values (R^2) obtained from different kinetic models, (Mean ±SD, n = 27)

The mean R^2 values of Zero-order, First-order and Hixon-Crowell models were found to be close to each other. Hence, two-sample t-tests between (i) Zero-order and First-order and (ii) Zero-order and Hixon-Crowell models were carried out with Minitab 18 software. In both cases, the difference between two means was found to be significant at 95% confidence level. Therefore, the R^2–value of Zero-order kinetics was found to be significantly highest among Zero, First and Hixon models. Therefore, it may be concluded that the release of acyclovir from the liposomes may be assumed to follow zero-order kinetics.

Drug stability study

Table 2: Drug stability study

Time	Batch	Color	Percentage of drug entrapment efficiency	t_{30} (min)	Changes in physical properties
1 mo	F_4	Yellow	62.38±5.24	101.44±7.65	No changes

(N=3, Mean ± SD)

Formulation F_4 was stored in three Eppendorf's tubes for one month. At the end of one month, samples were analyzed and the results are displayed in table-2. The values of drug entrapment efficiency and t_{30} were found to be altered insignificantly. Therefore, the liposomes may be assumed to be stable till one month.

DISCUSSION

Liposomes function as drug carriers. Liposomes formation took place when phospholipids were dispersed in the aqueous medium and due to hydrophilic interaction of the lipid head groups with water. [28] In this study, total nine batches of liposomal acyclovir were prepared and each batch repeated thrice. The formulation contained several excipients like Lecithin, Cholesterol and Chloroform (solvent). Liposomal formulation of acyclovir was prepared by "Thin Lipid Film Method". In this method sonication was a vital step for mixing up of all excipient, including the drug. Lecithin is a very dense excipient to be suspended properly without sonication. After proper mixing of all ingredients it was very important to dry the suspension in water bath with rotational movement, which would help in the formation of thin lipid film. The evaporation process was continued until the smell of chloroform was present. The freshly prepared suspensions were kept for overnight at 4 °C in refrigerator before any evaluation procedure.

Drug entrapment study was performed and better entrapments were found from batch F_2 and F_9 which gave drug entrapment efficiency respectively about 75.49±0.51% and 73.637±9.26%. Drug release study of all batches was performed by using phosphate buffer (pH 7.4) medium. After release study it was found that formulation F_4 showed higher percentage release (79.021 %) and F_1 showed second-highest percentage release (63.642 %) in 4 h. The release data was fitted to different kinetic models like Zero order, First order, Higuchi, Hixoncrowell and Korsmeyer-peppas. The highest correlation coefficients was found with zero-order model (r=0.9970) among all models. Hence, it may be concluded that the release of Acyclovir from liposomes follows zero-order kinetics and gave controlled drug release [29-31]. All nine batches followed zero-order kinetics these means that they all lies under control release drug delivery system. According to the kinetic study F_9 followed zero-order kinetics rather than other batches because they showed higher correlation coefficient compared to other formulations. That means these formulations followed a controlled release drug delivery system. From fig. 3 it is evident that F_4 (Cholesterol 32.9 mol%) showed lowest t_{30} value, in other words, the highest release rate among all other formulations. The lowest release rate corresponding to the highest t_{30} value was found from F_1 formulation containing 66.2 mol% of cholesterol. So it may be concluded that rate of release of Acyclovir took place with decreasing cholesterol mol%. Therefore, by controlling the cholesterol mol% the rate of release of acyclovir may be controlled from liposomal formulations. From the stability study results with F_4 formulation the drug entrapment efficiency and the release rate (exhibited by t_{30} value) changes were found after one month but those were insignificant.

CONCLUSION

The project was performed to exploit the activity of Acyclovir in the form of a liposome. Nine batches of a liposomal formulation of Acyclovir with varying proportions of cholesterol and lecithin were prepared and evaluated to estimate their entrapment efficiency, release rate, drug release kinetics and stability testing. Most of the batches were found to follow zero-order release kinetics. F_3 and F_9 showed lowest and highest drug entrapment efficiencies, respectively with a pattern of increasing entrapment efficiency with decreasing proportion of cholesterol in the lipid phase. From the t_{30}% drug release study it was founded that F_4 gave slowest drug release and F_9 gave the highest drug release rate. Therefore two or more formulations with varied release rates can be combined to produce a formulation with a required release rate. As F_4 shows highest release pattern in 4 h, the stability testing of this formulation was carried out and the formulation was found to be stable after one month's storage at 4 °C temperature. Though, long term stability study and clinical trial is required for future development of this dosage form.

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AUTHORS CONTRIBUTIONS

All authors contributed equally

CONFLICT OF INTERESTS

The authors declared no conflict of interest

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