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Design and Evaluation of a Lyophilized Liposomal Gel of an Antiviral Drug for Intravaginal Delivery

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ABSTRACT: Liposomes of antiviral drug(acyclovir) prepared by rotary evaporation method were incorporated into two bioadhesive polymers, carbopol and HPMC and freeze dried to obtain a unit dosage form. The liposomes, liposomal gels and freeze dried rods were evaluated for various parameters. TEM analysis showed the formation of unilamellar liposomes with a mean diameter ranging from 0.9 μm to 1.2 μm. As the cholesterol content increases from 0.5% to 2%w/w, the entrapment efficiency and vesicle size increased. Carbopol gels exhibited higher viscosity, spreadability, mucoadhesiveness than HPMC gels. The redipsersion of freeze dried forms in SVF was found to be slow and its *ex-vivo* retention time was found to be 12 hrs while acyclovir gel retained only for 8.25 hrs. The tablet and gel released 96.93±0.15% acyclovir within 6 hrs and 92.31±0.31% by 8 hrs respectively while freeze dried forms could sustain the release upto 12 hrs. From the stability studies the optimum storage condition was found to be 4-8°C. © 2013 Wiley Periodicals, Inc. J. Appl. Polym. Sci. **2014**, *131*, 39804.

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INTRODUCTION

The causes of sexually transmitted diseases (STDs) are bacteria, parasites, and viruses. There are more than 20 types of STDs, including chlamydia, gonorrhea, genital herpes, HIV/AIDS, syphilis, and trichomoniasis. STDs that are undiagnosed, untreated, and uncared for enhance the chance for HIV transmission. Most STDs affect both men and women, but in many cases, the health problems they cause can be more severe for women. To date, the treatment of these STDs is available in tablet, capsule, or ointment form of antiviral drugs such as acyclovir (ACV).

ACV, a synthetic analogue of 20-deoxiguanosine, is one of the most effective and selective agents against viruses of the herpes group. Unfortunately, its absolute oral bioavailability is considerably poor (ca. 15–30%) because of its low water solubility (ca. 0.2%, 25°C) and short half-life (ca. 2.5 h). Therefore, ACV must be taken in an oral dose of 200 mg five times daily, and this can cause compliance problems in patients.[1] Hence, this drug-delivery system was designed to deliver a drug effectively, specifically to the site of action and to minimize the toxic effects compared to those of conventional dosage forms. Intravaginal administration is an interesting alternative for reducing side effects and for increasing drug targeting and therapeutic benefits.

Liposomes are broadly defined as lipid bilayers surrounding an aqueous space.[2] Liposomes have been investigated as a potential vaginal delivery system because of their ability to provide the sustained and controlled release of an incorporated material. In general, they are deemed to be more effective and less toxic than conventional formulations because of their bilayer composition and structure.[3] Depending on the composition, liposomes can have a positive, negative, or neutral surface charge. If a drug is water soluble, it will be encapsulated within the aqueous compartment, and lipophilic drugs will usually be bound to the lipid bilayer or dissolved in the lipid phase.[4] Liposomal entrapment has a considerable effect on the pharmacokinetics and tissue distribution of the administered drugs. It is likely to reduce drug uptake in these selected areas by means of liposomal entrapment, and this may be a useful means for decreasing the systemic toxicity of drugs.[5]

Inducing therapeutic action by the vaginal instillation of liposomes containing an antiviral drug is far from ideal because of the liquid nature of the preparation, leakage, messiness, and low residence time; these will contribute to poor subject or patient compliance. Hence, there is strong rationale for expanding the formulation options for effective vaginal administration.[6] Liposomes can be formulated into gels with suitable mucoadhesive polymers, such as methyl cellulose

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Table I. Formulation Chart for ACV Liposomes

Formulation	Cholesterol (mg)	Soya lecithin (mg)	Cetyl pyridinium chloride (mg)
F1	500	250	—
F2	500	500	—
F3	500	750	—
F4	500	1000	—
F5	250	500	—
F6	750	500	—
F7	1000	500	—
F8	500	250	250
F9	500	500	500
F10	500	750	750
F11	500	1000	1000
F12	250	500	500
F13	750	500	500
F14	1000	500	500

(MC), Carboxy Methyl Cellulose (CMC), and hydroxyl propyl methyl cellulose (HPMC) bases, to take a full advantage of the contact time. Previous studies have reported the success of this novel delivery system in attaining the sustained release of ACV by intravaginal delivery.[7] Another recent study reported the reduced irritancy and slow release of ACV from a liposomal gel through the epidermis.[8] However, the direct application of gels onto infected sites of the vagina might be difficult and inconvenient due to leakage, and it might require frequent dosing because conventional gels do not remain for a long time at the site of application.

Because of its initial success, we took research efforts to next level in this work by a comparative study of different polymeric liposomal gels. Furthermore, a novel attempt has been made to obtain a unit dosage form of liposomal gels by freeze drying, as it offers a stable and practical method for easy application. This study was designed to attain a sustained release of ACV by the intravaginal delivery of a freeze-dried liposomal gel instead of by repeated oral administration of tablets.

EXPERIMENTAL

Materials

ACV was obtained as a gift sample from Micro Labs, Ltd. (Bangalore, India). Cholesterol, cetyl pyridinium chloride, sucrose, HPMC, and Carbopol 934 were obtained from Loba Chemie Pvt., Ltd. (Mumbai, India). Soya lecithin and the dialysis membrane (pore size = 2.4 nm, molecular weight cutoff = 12,000–14,000 Da) were purchased from Sigma-Aldrich (Mumbai, India). All of the other chemicals were reagent grade.

Preparation of pH 4.2 Simulated Vaginal Fluid (SVF)[3]

NaCl (3.51 g), KOH (1.40 g), $Ca(OH)_2$ (0.222 g), bovine serum albumin (0.018 g), lactic acid (2 g), acetic acid (1 g), glycerol (0.16 g), urea (0.4 g), and glucose (5 g) were dissolved in 1 L of deionized water. The pH of the solution was then adjusted to 4.2 with HCl.

Methods

Preparation of the ACV Liposome. Different compositions and differently charged liposomes were prepared by a dehydration–rehydration method. Required quantities of soya lecithin and cholesterol were placed, as indicated in Table I, into a round-bottom flask. They were dissolved in 50 mL of a solvent mixture made with chloroform and ethanol (8:2). For the preparation of positively charged liposomes, cetyl pyridinium chloride was also added to the solvent mixture. The round-bottomed flask was attached to a rotary flask evaporator and rotated at 80 rpm at 65°C until the solvent was evaporated; this left a thin residual lipid film on the wall of the container. The flask was then kept in a desiccator to remove any traces of organic solvent. To the film, a 1% sucrose solution containing the drug was added immediately, and the flask was rotated under similar conditions for 30 min until all of the lipid film entered the aqueous medium. This resulted in the formation of liposomes, which were identified by the formation of a milky white colloidal solution. The liposomes were allowed to swell overnight under refrigeration. All of the formulations contained 400 mg of ACV.

Formulation of the Liposomal Gel of ACV. The gel base was prepared by the dispersal of a required quantity of polymer (Carbopol 934 and HPMC) in distilled water with the help of a magnetic stirrer. The previous solution was kept overnight to

Table II. Ingredients Used in the Formulation of the Gel Base

Sample no.	Ingredient	Quantity
1	Gelling agent	1% Carbopol, 2% HPMC
2	Propylene glycol	0.25 mL
3	DMSO	0.1 mL
4	Sodium benzoate	0.1 mg
5	Ethanol	0.25 mL
6	Distilled water	Up to 50 mL

Table III. Formulation Chart for the Lyophilized Liposomal Gels of ACV

Formulation	Liposomal dispersion (equivalent to 400 mg)	Gel base (1 g)
G1	F4	1% Carbopol
G2	F11	1% Carbopol
G3	F4	2% HPMC
G4	F11	2% HPMC

completely hydrate the polymer. Dimethyl sulfoxide (DMSO) and ethanol were added to make the gel soft. Care was taken to prevent the incorporation of air into the gel. The pH of each formulation was adjusted in the range 3.8–4.2 (to be within the normal vaginal pH range) by the addition of 1% lactic acid.

From the prepared gel base, 1 g was taken, and a liposomal dispersion containing 400 mg of ACV was added to it with the help of a magnetic stirrer. The components, along with their concentrations, used for the preparation of liposomal gel are given in Table II. Plain ACV gel (4%), without any drug was also prepared with the same gelling agents for the comparative study of the retention times.

Lyophilization. The prepared liposomal gels were filled into rod-shaped molds and subjected to freeze drying (Table III). The gel was frozen to −40°C, and freeze drying was performed in a freeze drier (Ilshin Laboratory Co., Japan) until a soft mass was obtained. The whole drying process took 24 h with primary drying at −40°C for 16 h and secondary drying at −20°C for 8 h. Sucrose at a ratio of 1:1 with lipid was used as a cryoprotectant.

Fourier Transform Infrared (FTIR) Spectroscopy

A FTIR spectrophotometer (Shimadzu 8400S, Japan) was used to study the FTIR spectra by a KBr pellet method. The solid pellet was prepared with KBr at room temperature and then analyzed. An FTIR study was also performed on the formulation.

Differential Scanning Calorimetry (DSC)

All of the dynamic DSC studies were carried out with a differential scanning calorimeter (DSC 60, Shimadzu Corp., Japan). The thermogram of the pure ACV, physical mixture, and freeze-dried product were compared. The aluminum cuvette containing the sample was heated from 0 to 320°C at a heating rate of 10°C/min with nitrogen as a purge gas.

Drug-Entrapment Studies

The entrapment efficiency of the liposomes was determined by a centrifugation method. Aliquots (10 mL) of the liposomal dispersion were subjected to centrifugation on a laboratory centrifuge (Remi R4C) at 10,000 rpm at a temperature of 4°C for 30 min. The clear supernatant was removed to separate the nonentrapped ACV. The sediment of the liposomes was washed again with buffer to remove any unentrapped drug, and the washings were combined with supernatant. The resulting solution was analyzed for drug content at 253 nm after suitable dilutions. The following formula was used:

$$\text{Drug entrapment (\%)} = \frac{\text{Total drug added} - \text{Unentrapped drug}}{\text{Total drug added}} \times 100$$

Table IV. Standard Values for Spreadability[10]

Fluid gel	>0.24 cm
Semifluid gel	0.24–2.59 cm
Semistiff gel	<2.59–1.64 cm
Stiff gel	<1.64–1.4 cm
Very stiff gel	<1.4 cm

Vesicle Size Analysis and ζ Potential

The average vesicle diameter and ζ potential of the liposomes were determined with a Zetasizer instrument (3000HSA, Malvern Instruments, United Kingdom). The liposomal dispersion was suitably diluted, and the vesicle size was measured at 25°C.

Photomicroscopic Studies

The vesicle morphology of the liposomes was determined with a photomicroscope (Olympus, model CXX41) at 40× magnification. Transmission electron microscopy (TEM) analysis was carried out with a Morgegni 268D instrument (FEI, Holland). The samples were mounted on copper gridding, dried *in vacuo*, and scanned at an accelerating voltage of 15 kV before observation.

Evaluation of the ACV Liposomal Gels

The prepared liposomal gels were physically evaluated for homogeneity and grittiness. The viscosity was determined at 25°C with a Brookfield viscometer with number 94 spindle at 1.5, 20, 40, and 50 rpm (Brookfield Engineering Laboratories, model HADV-II, Middleboro, MA). We determined the spreadability was determined by taking 100 mg of the liposomal gels on one glass plate. Another glass plate was dropped from a distance of 5 cm. The diameter of the circle of spreaded liposomal gel was measured. The classification given in Table IV was adopted.

Mucoadhesion Studies[9]

Isolated goat vaginal tissue was cleaned and separated from the supporting tissues and then kept at 0°C until further use. Before

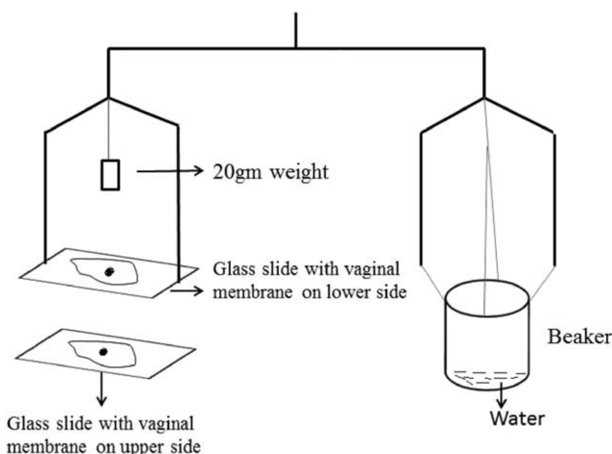

Figure 1. Schematic diagram illustrating bioadhesion measurement by the modified balance method.

the experiments, the goat vaginal tissue was thawed in normal saline. The bioadhesion measurement was performed with a modified balance (Figure 1). The two pans of the physical balance were removed. The right side of the pan was replaced with a 100-mL beaker, and on the left side, a glass slide was hanged. To balance the assembly, a weight of 20 g was hanged on left side. Another glass slide was placed below the hanged slide. Portions of the vaginal membranes were attached with both slides. The gel (1 g) was placed between two vaginal membrane faces. A little pressure was applied to form a bioadhesion bond, and then, water was slowly added on the right-side beaker until the gel was separated from one face of the attached vaginal membranes. The volume of water added was converted to mass. This gave the bioadhesive strength of the gel (grams).

Evaluation of the Freeze-Dried Liposomal Gel

Redispersibility. The redispersibility of the liposome-gel-based freeze-dried forms was evaluated in the SVF. The freeze-dried forms were placed in 2 mL of SVF and incubated at 37°C in an orbital shaker at 20 rpm for about 24 h. The ease of redispersibility was observed and ranked from 1 to 3, with 1 being completely redispersbile and 3 indicating the least redispersibility.

The gel obtained by redispersion was evaluated for the percentage of drug entrapment, viscosity, and mucoadhesion strength.

***Ex Vivo* Retention Measurement.**[11] For the effective vaginal delivery of an antiviral drug, it is highly recommended that the drug-delivery system should reside for a prolonged period of time. Hence, the retention times of the freeze-dried liposomal forms G1 and G3 were determined and compared with that of the plain ACV gel. An intact 5-cm tubular piece of goat vagina was procured from a slaughter house and was thawed in normal saline. The tissue was surrounded with a cotton pad moistened with normal saline and further surrounded by aluminum foil to keep the tissue moist. It was suspended in an inclined plane with the help of a loop of wire and a stand. A burette was set above the vaginal tube, from which pH 4.2 SVF was added to the tissue drop by drop throughout the experiment. This helped us study the influence of various vaginal fluids and its environment on the retention of the dosage form. An electronic balance was placed below the suspended tissue to measure the weight of the gel falling down. The test sample (1 g of freeze-dried rods/1 g of ACV gel) was introduced into the isolated vaginal tube. The residence time of the samples was determined as the time taken for the expulsion of the entire 1 g of gel.

***In Vitro* Drug-Release Studies.**[12] The release of ACV from the optimized formulation was studied with the permeation apparatus designed as described. A glass cylinder with both ends open, 10 cm in height, 3.7 cm in outer diameter, and 3.1 cm in inner diameter was used as the permeation cell. A dialysis membrane, which was soaked in 500 mL of SVF pH 4.2 at 37°C for 5 h was fixed to one end of the cylinder. Then, 0.5 g of ACV liposomal gel was taken in the cell (donor compartment), and the cell was immersed in a beaker containing 100 mL of pH 4.2 SVF as the receptor compartment. The entire surface of the cell was in contact with the receptor compartment, which was agitated with a magnetic stirrer, and a temperature of 37 ± 1°C was maintained. The samples (5 mL) of the receptor compartment were taken at a 60-min time interval over a period of 6 h and replaced with an equal volume of fresh buffer solution. The sample was analyzed for ACV at 253 nm against a blank with UV spectroscopy. The drug release of the optimized ACV liposomal gel was compared with the drug release of its corresponding ACV liposomes, ACV gel, and a commercial ACV tablet.

The release studies of the ACV liposomes were carried out in a 250-mL beaker containing 100 mL of pH 4.2 SVF placed in a 250-mL beaker at 37 ± 5°C. The beaker was assembled on a magnetic stirrer. A dialysis membrane was taken, and one end of the membrane was sealed. The liposome dispersion was filled in the dialysis membrane, and the other end was closed and suspended in the medium. Aliquots were withdrawn (5 mL) at specific intervals and filtered, and the apparatus was immediately replenished with the same quantity of fresh buffer medium. The dissolution study of the ACV gel was carried out in the permeation apparatus as described previously, and the study of a commercial tablet in USP type II dissolution apparatus was carried out in pH 7.4 phosphate buffer.

Stability Studies

The optimized formulation of the freeze-dried liposomal gel was packed in a screw-capped bottle, and stability studies were carried out for a period of 6 months at 4°C, 25 ± 2°C and 60 ± 5% relative humidity, and 40 ± 2°C and 75 ± 5% relative humidity. The samples were withdrawn at regular intervals and spectrophotometrically analyzed for drug-entrapment at 253 nm.

RESULTS AND DISCUSSION

In general, STDs caused by viral infections are extremely difficult to treat, and there are very few effective therapies. Antiviral therapy based on intravaginal delivery may be enhanced by various techniques. Among antiviral drugs, ACV is the most widely used. Because of its poor pharmacokinetic profile and the side effects caused by its overdosage, the development of a delivery system that can enhance the bioavailability of this drug and prolong its effects is in high demand.[3] Hence, an alternative dosage form that was beneficial over oral tablets and topical gels in the treatment of genital HSV infections in women was designed. An attempt was made to formulate liposomes as ACV, incorporate them into a gel base, and further lyophilize them to obtain a freeze-dried liposomal gel. Lyophilization was adopted to increase the stability and retention time.

Various batches of liposomes, F1–F13, were prepared through the variation of the ratios of soya lecithin and cholesterol, as shown in Table I. The ratios were selected to study the influence of cholesterol and phospholipid on the drug entrapment. Because a prolonged retention on the mucous wall is often required for the desired therapeutic effect, research efforts have been directed to the use of hydrophilic polymers with bioadhesive characteristics to improve the delivery of ACV liposomes via the vagina. It has already been proven that liposomes are compatible with polymers derived from crosslinked poly(acrylic acid) polymer and cellulose derivatives.[7] Research studies have also proven that liposomes, when incorporated into a hydrogel base, provide a sustained release of drug through vaginal mucosa and through skin.[8] Therefore, a gel made of 1%

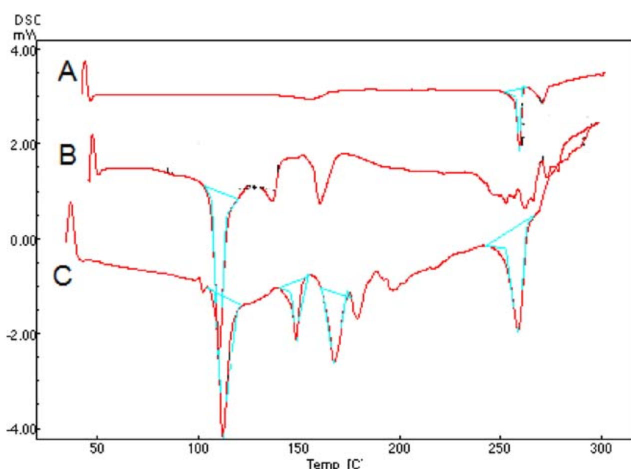

Figure 2. Thermograms of (A) pure ACV, (B) the formulation, and (C) the physical mixture. The aluminum cuvette containing the sample was heated from 0 to 320°C at a heating rate of 10°C/min with nitrogen as the purge gas. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

Carbopol 934 and 3% HPMC was chosen as a vehicle for liposome incorporation. The rationale for selecting concentrations of 1 and 3% was to attain a suitable viscosity that could enhance the retention of the freeze-dried rods in the vaginal cavity. The two polymers were compared in terms of drug release, rheological properties, and mucoadhesion strength. The pH of the vagina was maintained by lactobacilli, which produced sufficient lactic acid to acidify the vaginal secretions to pH 3.5–4.5.[9] Hence, the pH of all of the formulations was adjusted with lactic acid. On the basis of the results of the drug-entrapment studies, liposomal formulations F4 and F11, which contained a 1:2 ratio of soya lecithin and cholesterol, were selected to formulate the liposomal gel.

The prepared liposomal gels were freeze-dried to obtain a unique, unit solid dosage form of 1 g each, containing 400 mg of the drug. The drying time and temperatures were selected after several trial and errors, such that the product obtained was neither too soft, causing leakage, nor completely dried to a rough mass or powder. Any change in the liposomal drug entrapment or vesicle size due to lyophilization was studied.

FTIR and DSC Analysis

The DSC thermograms of the pure ACV, the physical mixture of the drug and excipients and freeze-dried formulation are represented in Figure 2. The DSC thermograms showed a melting endotherm of cholesterol at 148°C and soya lecithin at 112°C. Pure ACV showed a single melting peak at 259°C and started to degrade as it melted. No drug peak and no shift in the lipid peak were observed in the formulation thermogram; this suggested significant entrapment of the drug within the liposomal bilayer. The FTIR and DSC studies showed no chemical interaction of the drug with the polymers or cholesterol.

ζ Potential

A ζ value of ±30 mV was essential for effective stability and to inhibit aggregation. In this study, the ζ potentials for the positively charged liposomes and neutral liposomes were found to be 21.3 and −39.3 Mv, respectively. Cetyl pyridinium chloride, which is a cationic surfactant produced by a positive ζ potential for F8–F14 and a negative ζ potential for F1–F7, was attributed to the neutral charge of soya lecithin. It was observed that the ζ potential of the prepared liposomes had sufficient charge to inhibit the aggregation of the vesicles. The presence of a charge, along a bilayer surface, ensured repulsion among the various liposome lamellae, and this led to a higher aqueous phase entrapment.

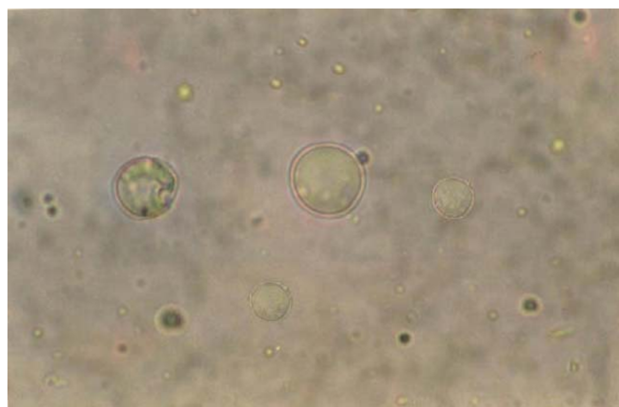

Figure 3. Optical photomicroscopy image of the prepared ACV liposomes at 40× magnification. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

Photomicroscopic Studies

Photomicroscopic studies with an Olympus model CXX41 and TEM studies showed the formation of several small, spherical, unilamellar vesicles of the ACV liposomes. The pictures are shown in Figures 3 and 4. The TEM images showed that the liposomes had a continuous and smooth surface; this indicated complete entrapment of the drug.

Vesicle Size Analysis

The vesicle size of the liposomes increased from 0.99 to 1.2 μm as the cholesterol was increased from 0.5 to 2%. The mean

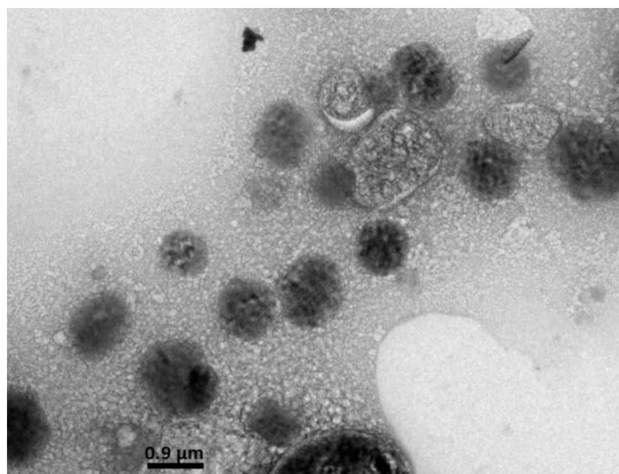

Figure 4. TEM image of the prepared ACV liposomes. The sample was mounted onto copper gridding, dried *in vacuo,* and scanned at an accelerating voltage of 15 kV before observation.

Table V. Vesicle Size Values for the Different Formulations

Formulation	Vesicle size (μm)
F2	0.99
F3	0.93
F4	1.20
F9	1.33
F10	1.75
F11	1.94
F4D	0.91
F4L	1.16

vesicle sizes of the empty (F4D) ACV liposomes and drug-loaded liposomal dispersion F4, both prepared under identical conditions, were 0.9 and 1.2 μm, respectively. This clearly indicated the entrapment of the ACV molecules in the liposomes. The bulky size of ACV was thus responsible for the increased mean diameter of the drug-loaded liposomes. The freeze-dried liposomal dispersion F4L showed a vesicle size of 1.16 μm compared to the normal liposomal dispersion F4, which had a vesicle size of 1.2 μm. This indicated that the use of the cryoprotectant prevented large differences in the vesicle size of the liposomes when they were subjected to lyophilization. The polydispersity index of the investigated formulations was in the range 0.219–0.293. A small polydispersity index value of less than 0.3 indicated homogeneous vesicle distribution. The results are represented in Table V.

Drug-Entrapment Studies

The percentage of drug entrapment of the ACV liposomes F1–F14 are presented in Table VI. From the results of the percentage of drug entrapped, we observed that as the ratio of lecithin to cholesterol was increased from 1:0.5 to 1:2, the entrapment efficiency also increased. Further increases in the cholesterol content resulted in a decrease in the entrapment efficiency. The results also show that as the concentration of phospholipid decreased, the drug-entrapment efficiency of the liposomes decreased. The entrapment efficiency decreased from 46 to 37% as the ratio of soya lecithin to cholesterol was reduced from 2:1 to 0.5:1. The positively charged liposomes showed a higher encapsulation compared to the neutral liposome formulation. From the results of drug entrapment, formulations F4 and F11, which entrapped the highest amounts of drug, were optimized to be formulated as ACV liposomal gels.

Table VI. Drug Entrapment Values for the Different Formulations

Formulation	Drug entrapment (%)	Formulation	Drug entrapment (%)
F1	45.21	F8	47.01
F2	54.45	F9	53.11
F3	55.56	F10	56.38
F4	68.37	F11	69.18
F5	28.34	F12	37.08
F6	39.51	F13	49.01
F7	43.92	F14	46.85

Effect of Cholesterol on Drug Entrapment. Cholesterol improves the fluidity of the bilayer membrane and improves the stability of bilayer membranes in the presence of biological fluids such as blood and plasma.[2] From the results of the percentage of drug entrapped, we observed that as the ratio of lecithin to cholesterol was increased from 1:0.5 to 1:2, the entrapment efficiency increased from 45 to 68%. Cholesterol is known to increase the rigidity of the liposomal membrane. This resulted in more rigid liposomes containing cholesterol during the incorporation process. Hence, there was a subsequent increase in the stability and rigidity of the liposomes. Furthermore, cholesterol made the membrane more ordered and prevented any phase transition of the liposomal system; hence, it was able to effectively prevent leakage of the drug from the liposomes. A further increase in the cholesterol content resulted in a decrease in the entrapment efficiency because higher amounts of cholesterol may have competed with the drug for packing space within the bilayer. The decrease in the entrapment efficiency with increasing cholesterol ratio above a certain limit may also have been due to the fact that an increase in the cholesterol amount beyond a certain concentration could have disrupted the regular linear structure of the vesicular membranes.

Effect of the Phospholipid on Drug Entrapment. The results of the drug-entrapment efficiency of the liposomes indicate that as the concentration of phospholipid decreased, the drug-entrapment efficiency of the liposomes also decreased. The entrapment efficiency decreased from 46 to 37% as the ratio of soya lecithin to cholesterol decreased from 2:1 to 0.5:1. This was due to the saturation of the lipid bilayer with reference to the drug, where the low phospholipid content was available for entrapment. This increase in liposomal drug levels with increasing lipid concentration could have been because, with increasing lipid concentration, the number of liposomes per milliliter of the formulation increased, and hence, there was more drug per milliliter of the formulation.

Evaluation of the Liposomal Gels

The viscosity, physical characteristics, pH, and spreadability of the liposomal gels G1–G4 were evaluated and are reported in Tables VII and VIII. It was evident from the results that in the

Table VII. Viscosity Values for the Formulations

Formulation	Viscosity (cps)			
	1.5 rpm	20 rpm	40 rpm	50 rpm
G1	Error	51,245	49,734	49,027
G2	Error	51,190	49,779	49,113
G3	229,000	42,431	40,231	39,088
G4	229,000	42,427	40,227	39,113
1% Carbopol gel base	Error	46,324	44,523	43,956
3% HPMC gel base	198,000	38,546	35,890	33,145

Table VIII. Evaluation of the Prepared Liposomal Gels

Evaluation parameter	G1	G2	G3	G4
Homogenity	Good	Good	Good	Good
Grittiness	—	—	—	—
Spreadability (cm): Mean ± SD[a]	1.26 ± 0.05	1.23 ± 0.15	1.53 ± 0.05	1.51 ± 0.15
pH	3.99	3.89	4.1	3.92
Mucoadhesive strength (g): Mean ± SD[a]	53.45 ± 0.05	52.92 ± 0.15	46.04 ± 0.05	46.11 ± 0.15

[a] SD, standard deviation. $n = 3$

Table IX. Results of the Redispersibity Test for the Formulations

Evaluation parameter	G1	G2	G3	G4
Ease of redispersion	2	2	1	2
Drug entrapment (%)	68.12	68.95	68.32	69.14
Viscosity at 20 rpm (cps)	47,214	47,356	42,190	42,241
Mucoadhesion strength (g): Mean ± SD[a]	51.35 ± 0.25	50.99 ± 0.15	45.91 ± 0.15	46.01 ± 0.15

[a] Standard deviation (SD), $n = 3$.

case of formulations containing Carbopol as a gelling agent, a higher spreadability and bioadhesion capacity was observed compared to that of the HPMC gels. The spreadability results indicate that the Carbopol and HPMC gels belonged to the very stiff and stiff gels categories, respectively. The difference in spreadability may have been due to the various viscosities of the gelling agents and the concentrations used. However, in the case of vaginal gels, the presence of a high viscosity or stiff gel not only speaks in favor of better efficacy but also increases the mucoadhesivity and retention of the gel formulation in the vaginal canal. The viscosity of the samples decreased with increasing rotational speed. Despite their relatively high viscosities, the freeze-dried forms were found to be easily redispersible. It was interesting that after the incorporation of liposomes into the gel base, there was an increase in the viscosity. Hence, a still lower concentration of gelling agent was also used in the formulation of the liposomal gel. The differences in the mucoadhesive strength between the Carbopol gels and HPMC gels could have been related to the differences in the polymer chain flexibility, the ability to form hydrogen bonds, and/or the extent of

Table X. Cumulative Drug Release from Formulations G1-G4

	Drug release (%)			
Time (h)	G1	G2	G3	G4
0.5	1.5 ± 3.67	2.8 ± 3.72	7.6 ± 4.13	8.2 ± 5.71
1	7.3 ± 3.04	9.3 ± 4.11	11.3 ± 4.01	18.6 ± 5.64
2	16.8 ± 4.25	14.1 ± 4.26	24.7 ± 4.68	24.9 ± 4.1
3	21.8 ± 2.69	25.5 ± 2.89	31.32 ± 5.04	33.6 ± 5.02
4	34.4 ± 5.06	37.6 ± 3.67	36.9 ± 3.71	40.32 ± 4.11
5	43.4 ± 4.15	44.7 ± 5.04	48.8 ± 2.14	47.8 ± 4.23
6	45.6 ± 3.76	47.2 ± 2.97	56.4 ± 2.01	55.7 ± 3.97
7	53.4 ± 5.02	55.9± 2.69	61.1 ± 2.67	59.7 ± 2.79
8	56.5± 2.69	59.2± 2.69	68.5 ± 4.26	66.3 ± 5.23
9	61.6 ± 5.04	64.7 ± 2.01	74.0 ± 2.01	72.4± 4.25
10	67.4 ± 4.26	68.2 ± 3.71	77.3 ± 2.04	79.2 ± 2.19
11	73.5 ± 3.72	72.0 ± 4.26	84.2 ± 3.72	84.9 ± 2.01
12	77.4 ± 0.11	79.5 ± 0.15	88.4 ± 2.01	89.2 ± 0.31

Standard deviation, $n = 3$

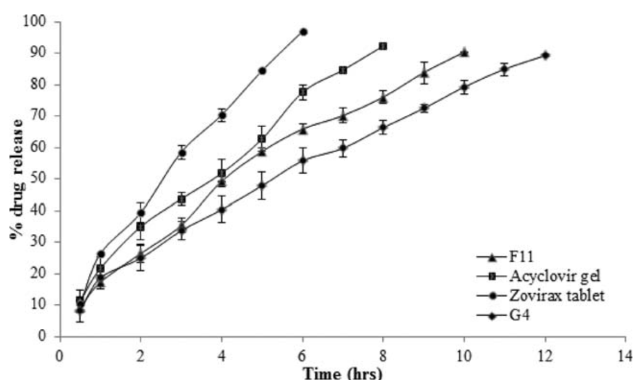

Figure 5. Comparative dissolution profiles. The dissolution of the freeze-dried liposomal gel G4, plain gel, and F11 liposomes was carried out with a dialysis membrane in pH 4.2 SVF. The dissolution of a 400-mg Zovirax tablet was carried out in a USP type II apparatus in a pH 7.4 phosphate buffer.

swelling of the polymers. In case of Carbopol, the presence of charged functional groups in the polymer chains may have rendered it as an anionic polyelectrolyte. In general, anionic polyelectrolytes have been found to form stronger mucoadhesive bonds compared to neutral polymers because of the formation of strong hydrogen bonds between the polymer functional groups and the mucosal layer.[13]

Redispersibility Test

The redispersion of the freeze-dried rods back to liposomal gels is a critical parameter that influences the drug-release pattern. The final formulation, that is, the freeze-dried ACV liposomal gel rods, was evaluated for its redispersibility. The properties of the gel formed after the dispersion of the freeze-dried rods in SVF are reported in Table IX.

We observed that the redispersion was not a sudden process but occurred slowly. This slow conversion of freeze-dried rods to liposomal gel helped to prolong the duration of action and to overcome the messiness/leakage caused by topical gels. The redispersed liposomal gel showed no characteristic change in the percentage of drug entrapped. However, the viscosity and mucoadhesive strength of the Carbopol gels (G1 and G2) were reduced; this might have been due to the swelling of the polymer in the presence of the electrolytes of SVF.

***Ex Vivo* Retention Measurement**

Conventional ACV gels, although generally perceived as safe, are associated with problems, including multiple dosing, no dose accuracy, leakage, and messiness, which causes discomfort to their users. These limitations lead to poor patient compliance and failure of the desired therapeutic effects. The retention of any vaginal dosage forms is influenced by various vaginal secretions and the self-cleansing action of the vaginal tract. To prove the longer residence time of the freeze-dried ACV liposomal gel in comparison to the ACV gel, the *ex vivo* retention time was determined with a goat vaginal tube.

We found that the G1 and G3 freeze-dried forms could be retained for 11.8 and 11.2 h, respectively, whereas the retention time of the ACV gel was 8.25 h. The much higher retention time of the freeze-dried forms in the goat vaginal tube when compared to that of the plain ACV gel was attributed to the high mucoadhesive strength contributed by the higher viscosity and polymer concentrations used. The *ex vivo* retention time determined proved that this novel delivery system was capable of delivering the drug for about 12 h, which would reduce the frequency of administration.***

***In Vitro* Drug-Diffusion Studies**

The *in vitro* drug diffusion of ACV from the liposomal gel formulations (G1–G4) are reported in Table X. From the results, we found that both the gels containing Carbopol and HPMC as a gelling agent had a sustained release. However, the Carbopol gels had a very slower drug-release rate, which exceeded the duration of action. The optimized liposomal gel (G4) of ACV was found to release about 28.03% of the drug at the end of 60 min, and about 94.2% of the drug was released at the end of 12 h.

The comparative profiles of the optimized formulation with its corresponding ACV liposomes, ACV gel, and commercial ACV tablet are shown in Figure 5. After 6 h of drug-release studies at 37°C in the SVF pH 4.2, more than 49% of the originally entrapped ACV was still retained in the liposomal gel, in comparison with 34% in liposomes. The marketed ACV tablet released $96.93 \pm 0.15\%$ of the drug within 6 h, and the ACV gel released $92.31 \pm 0.31\%$ at the end of 8 h, whereas the freeze-dried liposomal gel of ACV could sustain drug release for up to 12 h.

The sustained release behavior of G1–G4 occurred because, first, the drug encapsulated in the liposomes showed sustained release through the lipoidal bilayer, which was evident from the release profile of F11. Both the positively charged and neutral ACV liposomal gel were favorable toward the release of drug in the acidic environment of the vagina.

Second, the incorporation of liposomes into polymeric gelling agents also enhanced the sustained action of the drug from 10 to 12 h. In a comparison, the Carbopol gels had a slower drug-release rate than the HPMC gels. This decrease in the release could be attributed to the increased microviscosity of the Carbopol gel. The viscosity was negatively related to the release of active substance from the formulations and its penetration through the diffusion barriers.[12] The swelling index of the polymers also influenced the drug-release behavior of the gels. We found that the gelling agent with a higher swelling index retarded the release of the drug more than those with lower swelling indices.[14] HPMC was reported to have a very low swelling index when used at a concentration of 3%. This swelling also depended on the pH of the medium and electrolytes, where the pH 4.2 SVF had a lot of electrolytes, such as sodium chloride, potassium hydroxide, and calcium hydroxide. Furthermore, the low swelling index of HPMC caused a low viscosity; this, in turn, explained the higher drug release. With the retention time of 12 h, which was determined by *ex vivo* testing, the formulation G4, which released 89% within the retention time, was optimized. Thus, formulation G4, which showed sustained

action and could release a maximum amount of drug within the retention time, was optimized.

The designed formulation was a unit freeze-dried rod of ACV liposomal gel that contained 400 mg of the drug. Unlike the topical gels, which failed to deliver an accurate amount of drug, this novel delivery system delivered an accurate dose of the drug on a single use just as an oral tablet or capsule. Hence, the release profile was compared with that of an oral ACV tablet.

In vitro release studies proved that it was capable of delivering the drug for a longer period of time compared to the oral tablet and topical gel.

CONCLUSIONS

The prepared freeze-dried rods of ACV liposomal gels overcame the major challenges of delivering antiviral drugs through the vaginal route for the treatment of STDs. The bilayer entrapment and the polymeric gelling agent sustained the release of the drug and thereby prolonged the duration of action. The liposomes prepared by rotary evaporation with phosphatidyl choline and cholesterol were unilamellar with vesicle sizes in range 0.9–1.2 μm. The incorporation of liposomes in bioadhesive gels improved their stability. The Carbopol gels were found to have a higher viscosity, spreadability, mucoadhesive strength, and *ex vivo* retention time than the HPMC gels. The freeze-dried rods were highly efficient as unit, stable, and in a comfortable dosage form for easy application that could deliver an accurate amount of drug. The use of cryoprotectant prevented structural changes in the liposomes during lyophilization. Although all of the formulations showed a sustained action, the HPMC gels with a 1:2 ratio of positively charged ACV liposomes, which showed sustained action but could release the maximum amount of drug within the retention time, was optimized.

Thus, the high viscosity and mucoadhesive strength imparted by the polymer and liposomes led to an increase in the retention time. The slow repdispersion and higher retention time reduced the dripping, leakage, and messiness caused by conventional gels. Furthermore, it was capable of delivering accurate doses of the drug for a longer period of time when compared to the oral tablet and thus minimizes the need for repeated administration.

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