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Research Article

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A Novel Vesicular Lipid Carrier Systems to Enhance Acyclovir Delivery through Skin

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ABSTRACT

There has always been a growing need for novel drug delivery systems to deal with chemical drug entities that have poor solubility and permeability. A great deal of interest has been being focused on the utilization of lipid based drug delivery systems such as ethosomes to enhance the permeation of antiviral drugs through the skin. Ethosomes have been employed to improve the permeability of drugs through different mechanisms. Acyclovir can pass easily through the skin to reach deep dermis layer where the virus replicates thus, enhancing the overall effect of the drug. Ethosomes comprise of various types of phospholipid structures, water, and low molecular weight alcohol (ethanol or isopropyl alcohol) in high concentration that provide malleability to the vesicle membrane. Hence, ethosomes loaded of Acyclovir were prepared with a purpose of overcoming these drawbacks. Ethosomes loaded sustained release formulations of Acyclovir were prepared using the hot method technique. The proposed formulations of Acyclovir loaded ethosomes were characterized for their morphology, particle size, zeta potential and entrapment efficiency and in vitro release study. The morphology of ethosomes showed ideal particle size and appearance. The entrapment efficiency was between 94.95 % and 98.56%, and the particle size was found between 276.3 nanometers to 677.7 nanometers. The porous structure of ethosomes was confirmed by an optical microscope and a transmission electron microscope. It also showed the highest release profile with nearly 28% release after 8 hours. F1 exhibited a zeta potential score of -67.8 indicating good stability. F1 in-vitro permeation study showed a promising penetration enhancement.

Key words: *Acyclovir, Ethosomes, Transmission Electron Microscope, In Vitro-Drug Release*

INTRODUCTION

Herpes simplex also known as HSV, is a common infection which has been well recognized globally, and it comprises of two main types : HSV1 and HSV2 in which the first actually is involved with facial or oral symptoms, and the latter causes genital symptoms. The disease is not considered life threatening until left untreated. It can be diagnosed through various examinations for example, physical examination, HSV swab test etc.., majorly the disease is usually a major concern for patients as it causes blisters, lesions and eye infections which urge patients to ask for treatment as they prefer to reduce the duration of infection and symptoms [1].

In case of eye infections, it can be dangerous for patients to leave the symptoms untreated as they could lead to loss in vision in one or both eyes (Fig.1). Herpes can be transmitted through absolutely normal day to day contact something as simple as physical contact to a lesion. The disease is usually managed with topical and systemic anti-viral therapies to limit outbreaks and lower the duration of infection as in the appearance of lesions or blisters, etc [2].

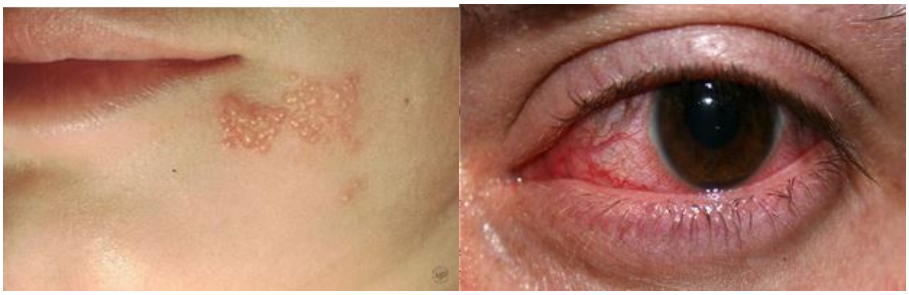

Figure 1 : HSV-1 face lesion and eye symptoms

A lot of people get the viral infection, however they don't feel or see anything. The signs that can be perceived by a person include : burning, tingling or itching ; mainly the skin may itch, burn or tingle for some time before the blisters start to appear. Symptoms similar to the flu can be observed including : muscle ache, fever, swollen lymph nodes in the groin or neck. Sores of fluid filled blisters that appear could break, often causing an oozing fluid and formation of a crust before healing. The first time they appear, they will continue to appear from two up to twenty days after contact with an infected person ; however they persist for a week or ten days according to the type : HSV1 (oral) or HSV2 (Genital). Herpes keratitis is an eye infection in which the virus can spread to both eyes ; however if this happens, you may experience photophobia, discharge, pain and a gritty sensation in the eye ; if left untreated scarring would occur and could lead to cloudy vision or even the loss of vision. Urination problems occur most often in women with genital type of the virus as they will experience a burning sensation and trouble while urinating [2].
If the person infected with HSV1 gets exposed to sunlight too much as in bad sunburns, it can worsen the case by triggering the herpes simplex virus like manipulating it [1].

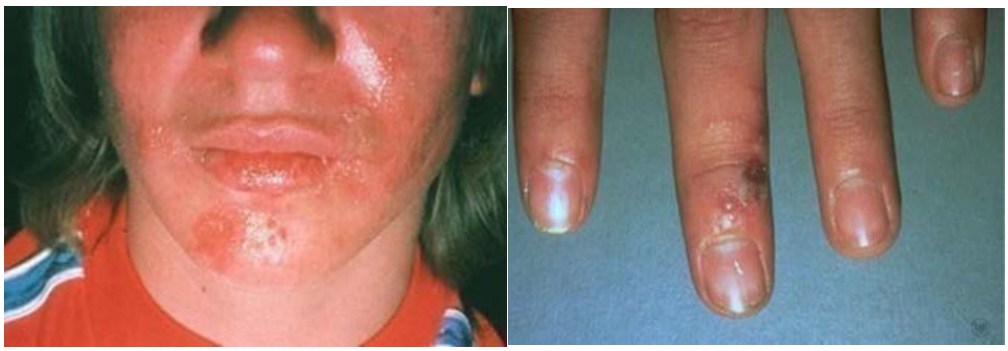

Figure 2 : HSV-1 face lesion and finger symptoms

Figure 2 shows HSV-1 face lesion and finger symptoms. There has been no cure for the herpes simplex virus in which the treatments target the cycle of the virus as ridding in the body of sores and limiting the outbreaks. The sores usually get clear without treatment, although people mostly choose the treatments for the infection to shorten the outbreak, and relieve the symptoms. Patients are usually prescribed with anti-viral medications in the form of cream or ointment applied topically in order to relieve tingling, itching and burning, while pills or injections usually indicated to target outbreaks of the virus. The following anti-viral drugs have been indicated including : Acyclovir (Zovirax), Valacyclovir (Valtrex) and Famiciclovir (Famvir). These anti-viral drugs can reduce the frequency and severity of outbreaks ; they can also help in the prevention of spreading the virus by infected people. The first outbreak is usually the worst, but with time the severity and frequency decrease due to the production of antibodies thus, the outcome will be good [1].

Acyclovir is a synthetic purine nucleoside analogue with in vitro and in vivo inhibitory activity against human herpes viruses, including herpes simplex virus (HSV) types 1 and 2, Varicella zoster virus (VZV), Epstein Barr virus (EBV), and Cytomegalovirus. The inhibitory activity of acyclovir for HSV 1 and HSV 2, VZV, EBV and CMV is highly selective. The enzyme thymidine kinase (TK) of normal and non-infected cells does not use acyclovir effectively as a substrate, hence toxicity to mammalian host cells is low ; however, TK encoded by HSV, VZV and EBV converts acyclovir to acyclovir monophosphate, and a nucleoside analogue which is further converted to the diphosphate and finally to the triphosphate by cellular enzymes. Acyclovir triphosphate interferes with the viral

DNA polymerase, and inhibits viral DNA replication with resultant chain termination following its incorporation into the viral DNA [3]. Figure (3) represents the mechanism of action of acyclovir.
The present study was aimed towards the development of controlled release formulations of acyclovir based on nano lipid carriers' ethosomes.

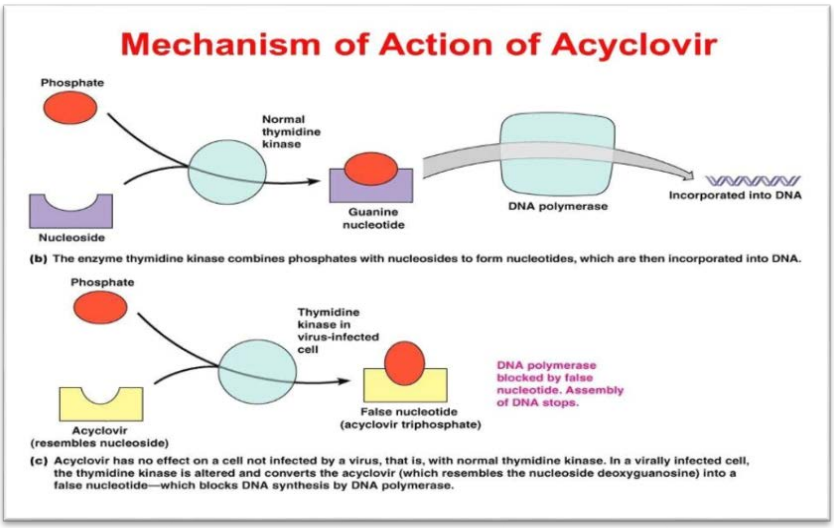

Figure 3 : represents the mechanism of action of acyclovir

Ethosomes are novel carrier system used for delivery of drugs having low penetration through the biological membrane mainly skin. Ethosomes are the slight modification of well-established drug carrier liposome [4]. Ethosomes are soft vesicles made of phospholipids and ethanol (in higher quantity) and water. The size range of ethosomes may vary from tens of nanometers to microns (μ) [5].
Ethosomes are easily prepared and considered as an economic drug delivery system as their constituents are easily acquired and handled. Vesicular drug delivery systems target the delivery rate that the body requires for treatment ; they control and target the delivery of drugs. They were developed by Paul Ehrlich in 1909 in which their basic use was delivery of the drugs to the diseased cells [6]. Figure (4) presents the structure of ethosomes

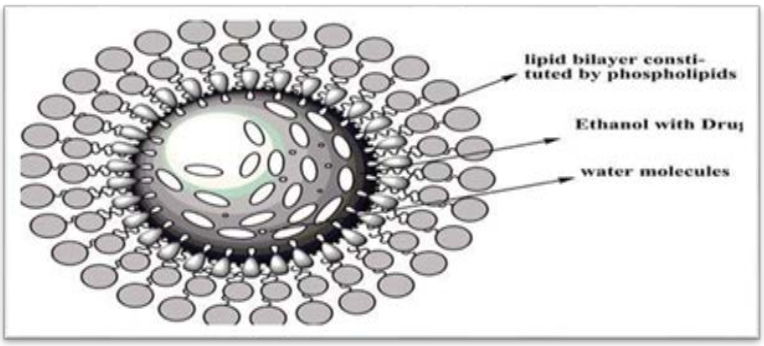

Figure 4 : Structure of ethosomes

MATERIALS AND METHODS :

Materials : Acyclovir was obtained as a gift from Novartis, Cairo, Egypt. Cholesterol, Sigma Chemicals, Soya bean lecithin, Sigma Chemicals were obtained from St. Louis, MO, U.S.A. Ethanol and Propylene glycol were obtained from SD Fine chemicals in Mumbai. All the other chemicals and reagents were of analytical grade.

Methodology :

1. **Calibration curve of Acyclovir in ethanol :**

The serial dilution of stock solution of Acyclovir in ethanol was used as a solvent ; Spectrophotometric analysis of each sample was done separately using ethanol as a blank. Calibration curve was obtained by the plotting of absorbance against the concentration. Linear regression analysis was done to show the least square line.

2. Determination of saturated solubility of Acyclovir in ethanol :

The preparation of a supersaturated solution of Acyclovir in ethanol was carried out using known excess of Acyclovir enough to give a supersaturated solution of 5 ml at a temperature of 25±1°C., in 48 hrs shake on electric shaker followed by 2 hr to settle [7].

3. The construction of standard calibration curve of acyclovir in the release media :

In accordance with the preliminary studies, acetate buffer with pH of 5.5 was chosen as the release medium for the ethosomal formula. The standard calibration curve of acyclovir in the freshly prepared acetate buffer of pH 5.5 was constructed with the same steps as mentioned before in the construction of the standard calibration curve of acyclovir in ethanol. The standard calibration curve was drawn through plotting the measured absorbance versus the concentration in which the least square line was obtained from the linear regression analysis [7].

4. Determination of saturated solubility of Acyclovir in acetate buffer as a release media :

The same procedures for saturated solubility of Acyclovir in ethanol were carried out except for the use of acetate buffer of pH 5.5 instead of ethanol, which was used as a blank. The concentration of the dissolved Acyclovir was obtained by the use of the previously constructed calibration curve of Acyclovir in freshly prepared acetate buffer of the same pH 5.5, this test was important for the determination of sink conditions of the release study which should be maintained at : $C1 < Cs \times 0.2$ (Cs : saturated solubility, C1 : the final concentration of Acyclovir followed a complete release in the release media).

5. Preparation of Ethosomes :

Hot Method :

Soya bean lecithin was dispersed in distilled water at about 40 ±1˚C. Acyclovir and cholesterol were dissolved in the mixture of propylene glycol and ethanol (2 :3 w/w). The organic solution was added slowly in a constant rate to the aqueous dispersion for 30 minutes under magnetic stirring at 750 rpm. Upon addition, the temperature of the aqueous dispersion was maintained at 40 ±1˚C. The prepared formula was stored at 4 ±1˚C. Three formulae were prepared through the previously mentioned techniques as shown in **Table (1)** [7].

Table 1 : Formulation chart of ethosomes containing acyclovir drug :

Formula code	Composition(total = 20gm)					
	Cholesterol W/W	Soya bean lecithin W/W	Ethanol	Propylene glycol	Acyclovir	Distilled water
F_1	1:	3	30%	20%	0.5%	q.s
F_2	1:	6	30%	20%	0.5%	q.s
F_3	1:	9	30%	20%	0.5%	q.s

Evaluation of the prepared ethosomal formulae :

1. Physical Examination :

All of the prepared formulae were inspected and assessed visually for their color, homogeneity, consistency, spreadability, and phase separation [8].

2. Optical microscopy observation :

The morphological aspects of the hot method of preparation of ethosomal vesicles were assessed by means of visual inspection and observation under an optical light microscope. Small quantities of the prepared formulae were placed on a clean glass slide with a slip cover followed by the observation at 40x magnification under room temperature conditions [9].

3. Tramission Electron Microscopy (TEM) :

Acyclovir loaded in ethosomes was visualized by using HR transmission electron microscope (JEOL JEM 2100, Japan). A drop of the formula was deposited on a copper 300- mesh grid, coated with carbon, and was allowed to stand for 10 minutes after which, any excess fluid was absorbed by a filter paper. Before examination, one drop of 1% phosphotungestic acid was applied and allowed to dry for 5 minutes [10].

4. Entrapment efficiency :

The entrapment efficiency of acyclovir ethosomal vesicles was determined through the ultracentrifugation method. One ml of each ethosomal formula was centrifuged for 60 minutes under 14000 RPM at ± 4°C. The vesicles were isolated through decanting the supernatant of un- entrapped acyclovir. The ethosomal formulas were lysed by sonication with a known amount of ethanol (which was clearly sufficient enough to solubilize acyclovir in accordance with the predetermined solubility) and the analyses of the drug content through UV spectrophotometry in which the amount of acyclovir entrapped within the vesicles was determined by the application of pre-fabricated calibration curve of acyclovir in ethanol. The entrapment efficiency was expressed as the percentage of the total

drug entrapped through using the following formula :

$$EE\% = C/T \times 100$$

In which T stands for the amount of drug that was added, while C represents the amount of drug detected after dissolving the vesicles [11].

5. Zeta potential determination and particle size analysis :

The Malvern Zeta sizer was used to determine the zeta potential of the selected formula. Dilution of the selected formula with distilled water was done in order to obtain a clear specimen for further analytic studies on the zeta sizer. The zeta potential was determined at ± 25°C using a clear disposable zeta cell in which the measurements took place in duplicates. The zeta sizer apparatus was used to analyze the vesicle size of formula in which 3 drops of the formula were diluted in distilled water and placed in zeta sizer cuvette followed by the blank cuvette for particle size analysis. The measurement was done in duplicates for both zeta potential and particle size analysis [7].

6. In vitro release study :

The in vitro release of acyclovir from all different formulae was carried out using the USP XXIV dissolution tester apparatus I and the rotating basket in the following order : 3 ml of each of the prepared formula was accurately measured, and placed in a silastic membrane bag (presoaked with the freshly prepared release medium acetate buffer of pH 5.5 overnight), it was well tightened and hanged up in the rotating shaft of apparatus I. The final concentration of acyclovir after the complete release in the medium was considered and maintained under the rules of sink condition. Bags were rotated at 100 rpm in a closed system (dissolution vessel was covered) that contained 100 ml of the freshly prepared release medium in which the thermostat was adjusted at a temperature of ± 32°C. At predetermined intervals (every 15 minutes during the first hour, every 30 minutes during the second hour and every 60 minutes for the following hours), 1 ml as specimen was withdrawn from the sink solution, and filtered and analyzed for acyclovir content through measuring the absorbance at predetermined wavelength using the release medium as the blank. Furthermore, a similar volume of the release medium was added as the compensation to the withdrawn volume in order to maintain the constant volume. Blank experiments were done at the same time of testing with application of the same procedures and conditions. The percentages of the released drug were calculated from the previously constructed standard calibration curve of acyclovir in the release medium acetate buffer with pH of 5.5 [12].

7. In vitro skin permeation studies :

The rate and extent of skin permeation of acyclovir from the gel was determined through franz cell diffusion apparatus. The cellophane membrane was soaked in buffer the day before the experiment, approximately 24 hours earlier. The weight of the sample that was 1 gm was withdrawn and placed inside the cellophane membrane, and was closed tightly. The volume of permeation media which was 7 ml (acetate buffer) was prepared. The temperature of the study was maintained at 32 °C. The sample was withdrawn at intervals of every 30 minutes, 2 ml for a total of 4 hours with volume compensation [10].

RESULTS AND DISCUSSION :

UV scanning of λ_{max} of acyclovir

Figure (5) presents UV λ_{max}scanning of acyclovir. The best absorbance for acyclovir was found to be 252 nm.

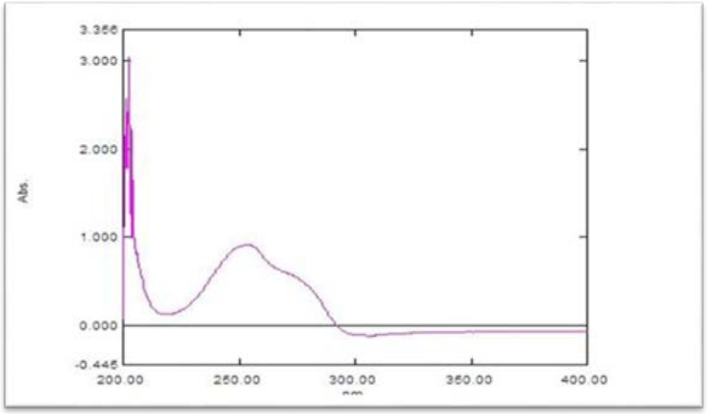

Figure 5 : UV λ_{max}scanning of acyclovir

Determination of the saturated solubility of acyclovir in ethanol :

The saturated solubility of acyclovir in ethanol was calculated to accurately determine the proper amount of ethanol that would lyse the ethosomal vesicles and dissolve its content, it was found to be more than 1 mg/ml [7]. Figure 6 and Table 2 represent the construction of standard calibration curve of acyclovir in ethanol.

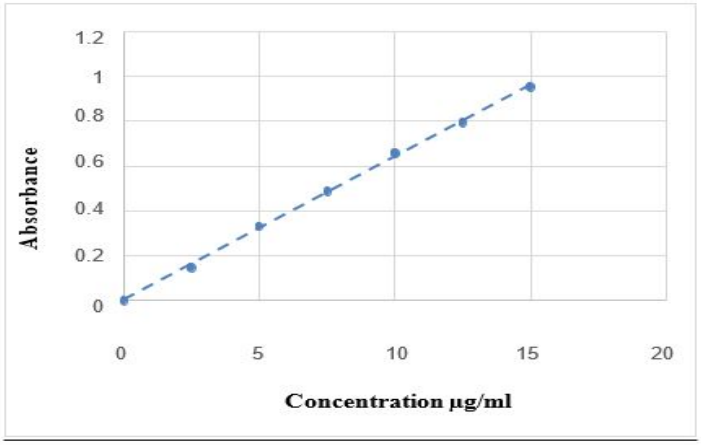

Figure 6 : Construction of standard calibration curve of acyclovir in ethanol

Table 2 : Construction of standard calibration curve of acyclovir in ethanol

Regression equation	R^2	K	Linearity Range
Y= 0.0639x+0.0012	0.9991	15.64595	2.5-15 µg/ml

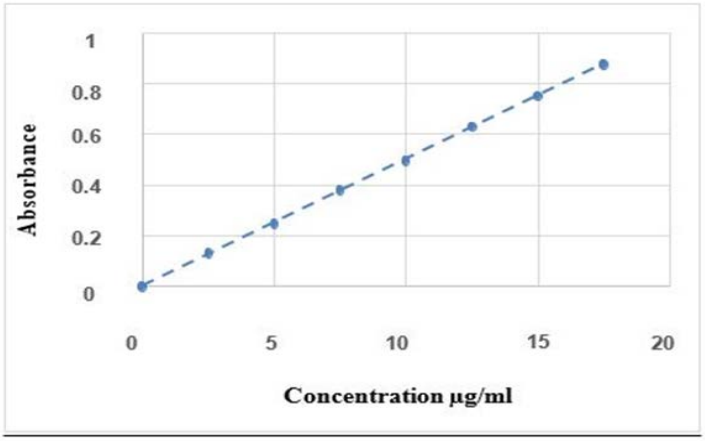

Figure 7 : Construction of standard calibration curve of acyclovir in acetate buffer

Table 3 : The Construction of standard calibration curve of acyclovir in acetate buffer

Regression equation	R^2	K	Linearity Range
Y= 0.0501x + 0.0004	0.9998	19.97337	2.5-17.5 µg/ml

Saturated solubility of acyclovir in the release medium (freshly prepared acetate buffer with pH of 5.5) was an important factor which may affect the acyclovir release study. The acyclovir saturated solubility in freshly prepared acetate buffer with pH of 5.5 was found to be 3.52 mg/ml. The sink conditions of acyclovir release study were considered to determine the used amount of the release media and/or the formula [7].

The present study was carried out to develop the Ethosomes of Acyclovir by the hot method. Hence, it was necessary to find suitable excipients with good compatibility. Six formulations of Acyclovir were prepared with different concentrations of Soya bean lecithin and Ethanol. Phospholipid was used as vesicle forming agent. Ethanol was used as a penetration enhancer. Propylene glycol was used as viscosity forming agent. Distilled water was used as a vehicle (Table.1).

Physical Examination :

All of the prepared formulae showed homogeneity in colour and appearance.

Optical microscopy observation :

Vesicle shape was determined by light microscope. It is shown in **Figure 8.**

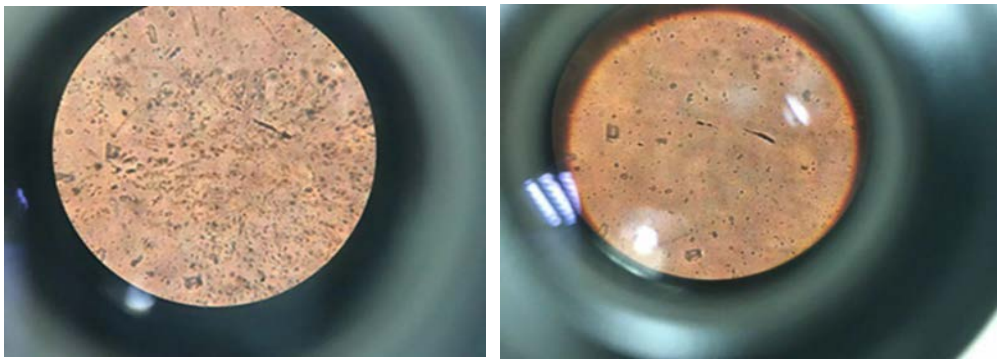

Figure 8 : light microscope visualization

Tramission Electron Microscopy (TEM) :

Transmission electron micrograph (TEM) of Acyclovir ethosomes dispersion illustrates the spherical shape of ethosomes entrapping the drug. The homogeneous monolayer coating of surfactant at the periphery of the nanoparticles surrounding the lipid core can be clearly seen in Fig 9.

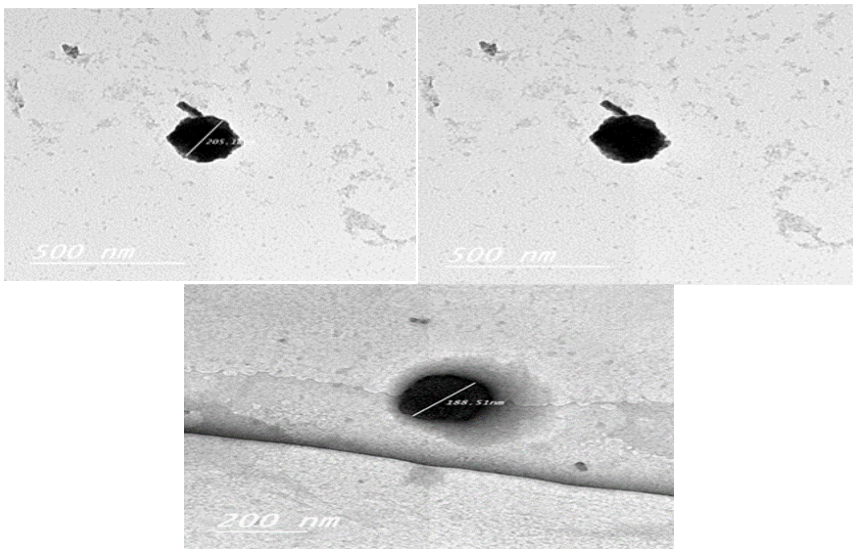

Figure 9 : Transmission electron microscope

Acyclovir EE% :

The entrapment efficiency of ethosomal vesicles of acyclovir was determined for all the formulae. The formulae were prepared with different cholesterol to soya bean lecithin ratios starting with F_1 1 :3, F_2 1 :6 and F3 1 :9. Cholesterol provided great rigidity to the lipid layers, and a higher stability. It reduced the vesicular fusion probability in high ultracentrifugation rotational energy through resistance. The formulae EE% were pretty close as most of the formulae exhibited more than 94% EE which was excellent ; however, F1 had the highest value which was one of the reasons why it was chosen [11]. **Table 4 represents the entrapment efficiency of Acyclovir :**

Table 4 : The entrapment

Formulae	EE%
F1	98%
F2	94.7%
F3	95.6%

In vitro release study :

In vitro release study of acyclovir from the ethosomal formulae was conducted in freshly prepared acetate buffer

with pH of 5.5 at 37°C ±1, the study was carried out in a period of 8 hours in compliance with the sink conditions. The percentage of acyclovir release was determined spectrophotometrically at λ_{max} 252 nm [7]. **Table (5) represents the percentage of drug released from the ethosomal formulae, and Figure 10 shows F_1, F_2 and F_3 release profiles.**

Table 5. The percentage of drug released from the ethosomal formulae.

F / Time	Release Percentage %		
	F_1	F_2	F_3
15 min.	10.7%	6.466%	9.3%
30 min.	13.4%	8.2%	10.733%
45 min.	14.8%	10.4%	11.933%
60 min.	15.03%	11.17%	13.166%
90 min.	15.667%	11.56%	13.833%
120 min.	16.83%	11.9%	14.06%
180 min.	18.2%	12.8%	14.1%
240 min.	21.3%	15.83%	15.03%
300 min.	20.6667%	16.93%	16.36%
360 min.	20.733%	18.766%	16.5%
420 min.	24.6667%	20.03%	18.2%
480 min.	28.6%	20.566%	21.566%

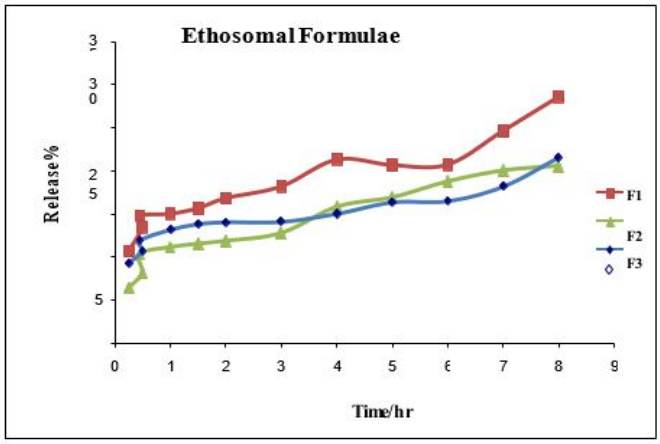

Figure 10 : F_1, F_2 and F_3 release profiles

It was observed that the drug release from the different ethosomal formulations had shown slow drug release during the first three hours [7]. The permeation of unentrapped drug from the aqueous solution was done in which the effect of the synthetic membrane was considered the main factor that controlled the release ; hence it was clear that the lower content of unentrapped drug or high EE% of the formulae caused lower release rate in the first three hours and vice versa. The ethosomal formulae drug release was slow, steady and decreased as a function of time. Better stability and high EE% formulae gave a prolonged release to the dispersions [7].

In vitro skin permeation study :

F_1 was chosen as the formula to continue further examination, as it showed the highest EE% and dissolution rate. The absorbance of each interval was measured, and the slope in acetate buffer was calculated through the standard calibration curve of acyclovir in acetate buffer which equaled = 0.050067. The K was calculated as it was the inversion of the slope as in 1/slope which equaled = 19.97337 µg/ml. The concentration of drug was plotted against time in the following figure [1]. The concentration of drug permeating through the membrane at each interval was calculated through the following equation :

Permeation conc. = Absorbance x K x Volume of the buffer x Dilution factor x 100/ wt of sample. Figure (11) represents the in vitro permeation plot of F_1.

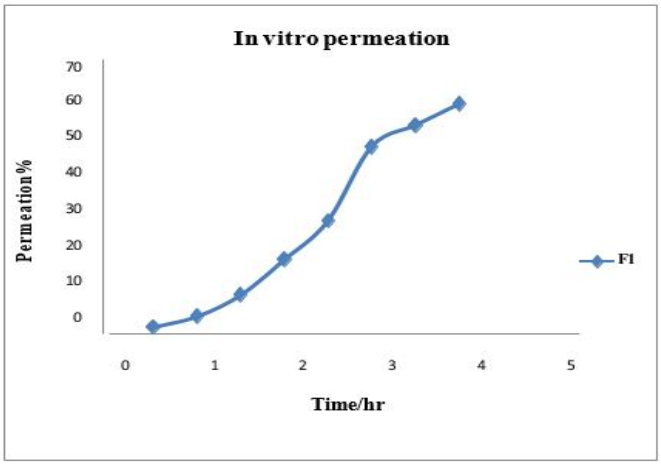

Figure 11. In vitro permeation plot of F1

The drug permeated the membrane slowly in the first hour, after that it increased and developed as a matter of time. This was owed to the fact that the vesicles were of high EE% which clarified why the drug was released slowly in the beginning of the test. The permeation results were very reasonable, and they did not achieve predictable results in terms of efficient permeation.

Zeta potential Determination and particle size analysis :

Zeta potential has been considered as a good indicator of ethosomal vesicular stability. Nanoparticles with zeta potential values from 0 to ± 5 were unstable (rapid coagulation/flocculation), from 10 to ± 30, they were of incipient instability, from 30 to ± 40 they were moderately stable, from 40 to ± 60, they were of good stability, and for more than ± 61, they were of excellent stability [13]. Dispersions with low zeta potential values were eventually aggregated as a result of van der waal interactions ; F1 the chosen formula exhibited -67.8 which indicated excellent stability [7]. Table 6 indicates Zeta potential score of F1. And, figure 12 shows the F1 Zeta potential plot.

Table 6 : Zeta potential score of F1

Formulae	Zeta potential score ±
F1	-67.8

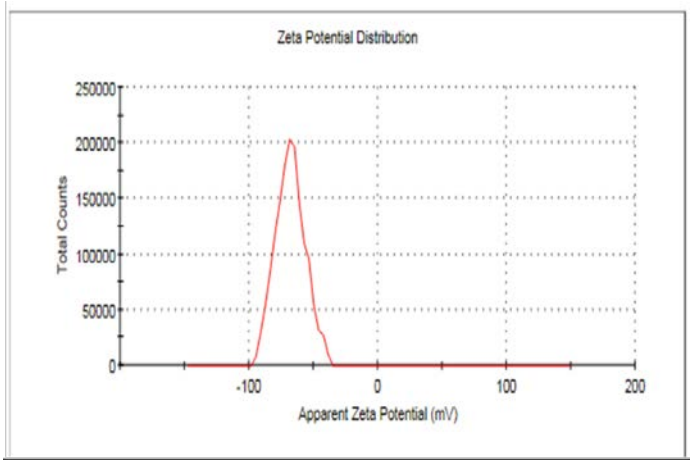

Figure 12 : F1 Zeta potential plot

The particle size of F1 vesicles was measured by the Malvern zeta sizer apparatus where the dilution by distilled water was necessary to obtain a clear solution for analysis. The particle size average in the first measurement was found to be 303.6 nm, while in the second measurement it was an average of 303.8 nm. The particle sizes were within the range according to the ethosomal nano-size range, and they were small which in turn yielded good surface area for absorption and thus, enhancing their applications. Figures 13 and 14

represent the F1 particle size plot in the first and second measurements ; respectively.

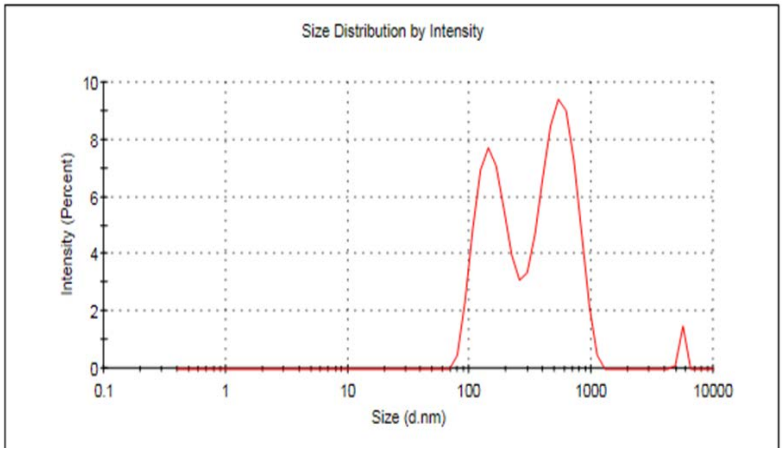

Figure 13 : F1 particle size plot

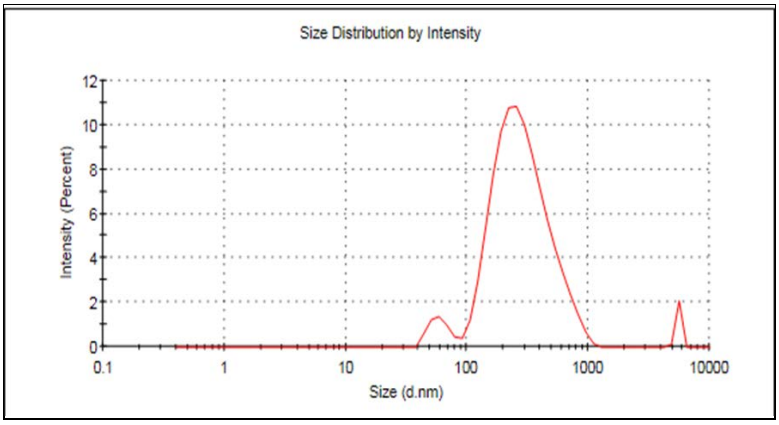

Figure 14 : F1 particle size plot

DISCUSSION

Ethosomes vesicles were prepared and loaded with acyclovir. The formulas were prepared by the hot method as mentioned previously to reach six formulae. All formulae were composed of the same composition and hydro-alcoholic-glycolic phospholipid concentration. The main difference between the formulae was the ratio of cholesterol to soya bean lecithin as mentioned previously in the materials used. Cholesterol was added to the lipid material for obtaining vesicular stability and its ratio was changed between the formulae in order to reach the best concentration with any of the three formulae. The basic combination of propylene glycol with ethanol yielded a synergistic potential that increased the rate of drug entrapment in the ethosomes. The ethanol propylene glycol combination was maintained within the range of what was recommended which was 22 to 70%. Ethanol caused the modification of the net charges of the system giving it some degree of steric stability which might ultimately decrease the size of the particles in other words : the mean particle size. The solubility of poorly soluble or insoluble dugs might be enhanced or increased when ethanol was used in higher concentrations in addition to enhancing the drug's entrapment inside the core of the vesicles. The recommended ethanol concentration ranged from 20 to 45% w/w in order to provide better entrapment efficiency, and furthermore increased the membrane's fluidity contrastingly. A much further increase in concentration was more than 45% which destabilized the vesicles, and made the vesicles leakier ; hence, decreasing the entrapment efficiency. The ethosomal vesicles were prepared with 30% ethanol which was sufficient for yielding high entrapment efficiency without negatively affecting the stability of the vesicles. When the ethanol system was stored, ethanol in the formula was volatilized easily, and it was easy to precipitate. Moreover, the low pH value inside the ethosomes and long period of incubation could exacerbate the stability of the lipid due to hydrolysis. On the PG basis, the volatilization of ethanol and ethanol system precipitation could be reduced. Therefore, PG 20% w/w was added to the ethanol content to

enhance the delivery of the drug and the stability. The concentration of phospholipids in the vesicles of ethosomes ranged from 0.5 to 10% w/w, and for cholesterol stability, it could be added at the concentrations of 0.1 to 1% w/w. Hence, the concentration of cholesterol usually ranged from 0.22 to 0.65%, while the concentration of soya bean lecithin was 2.14 to 2.5%. Visual inspection of the ethosomal formulae showed no remarkable difference between the three concentrations. All the prepared formulas had a characteristic color, and odour. The particle size ranged from118 nm to 637 nm, zeta potential showed great stability, and the entrapment efficiency of ethosomal vesicles of acyclovir was determined for all the formulae. The formulae were prepared with different cholesterol to soya bean lecithin ratios. Cholesterol provided great rigidity to the lipid layers, and a higher stability. It reduced the vesicular fusion probability in high ultracentrifugation rotational energy. It ranged from 89 % to 98 %. It was observed that the drug release from the different ethosomal formulations had shown slow drug release during the first three hours. The permeation of unentrapped drug from the aqueous solution was the effect of the synthetic membrane which was considered the main factor that controlled the release ; hence it was clear that the lower content of unentrapped drug or high EE% of the formulae caused lower release rate in the first three hours, and vice versa. The ethosomal formulae drug release was slow, steady, and decreased as a function of time. Better stability and high EE% formulae gave a prolonged release to the dispersions. Homogenous gel formulation was formulated with PH 5.6, no grittiness, high drug content and very good permeation release. The permeation results were very reasonable, they achieved predictable results in terms of efficient permeation. In the transmission electron microscope, the ethosomal vesicles appeared to have spherical morphology with imperfect round shapes in addition to different sizes. The observed round shapes assured the malleability of the vesicles.

CONCLUSION AND RECOMMENDATIONS

The ethosomal formulation was prepared easily, economically and successfully. The characterization of the vesicles was that they were spherical, homogenous, spreadable, transparent, and washable. The formulation had excellent stability zeta potential score which indicated no clumping. The release from the formulation satisfied the sustained release goal, while the permeation of the formulation satisfied the penetration enhancement goal. All in all, the ethosome nanoparticles were truly promising as a novel drug delivery system in terms of enhancing the penetration, so as sustaining the release of drugs, they were recommended as a system for the delivery of acyclovir through the skin. From the previous research papers on acyclovir, it could be concluded that acyclovir drug in nanoformulations can enhance the bioavailability to great extent, and they are highly absorbed inside the body

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