Proniosomes as a Stable Carrier for Oral Acyclovir: Formulation and Physicochemical Characterization

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Abstract: The purpose of the present investigation is to formulate and evaluate proniosomal carrier systems for Acyclovir. Acyclovir proniosomal delivery system was developed and subjected to *in vitro* and *ex vivo* studies. Acyclovir proniosomal carrier systems were prepared by three different methods using different carriers. Characterization of the prepared proniosomes and the proniosomes derived niosomes were also evaluated through particle size analysis, Microscopical examination, IR spectroscopy, DSC, *in vitro* dissolution and storage studies. Furthermore, *in vitro* release and intestinal permeation studies were tested and compared with drug solution. Results obtained showed that the prepared vesicles were spherical, discrete with no aggregation. Higher lipid concentration might have resulted in higher encapsulation volume and thus, increase of entrapment efficiency, in addition to an increase in the mean particle size. *In vitro* release studies pointed to an initial fast release of Acyclovir from proniosomes followed by relatively slower release up to 8 hours. Ex vivo intestinal permeability studies showed that drug diffused at a faster rate from the proniosome formulations than from drug solution. Stability studies suggested that proniosomes offer a more stable system that could minimize the problems associated with conventional niosomes. In conclusion, proniosomes may be a promising carrier for Acyclovir, especially due to their simple production methodology and stability.

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1. Introduction

Drug delivery system using colloidal particulate carrier (1, 2) such as liposomes or niosomes have distinct advantages over conventional dosage forms because the particles can act as drug containing reservoirs. Niosome, the surfactant vesicles, are spherical bilayered structures capable of entrapping water soluble solutes within an aqueous domain or alternatively lipid molecules within the lipid bilayers. Niosomes have been prepared from several classes of non-ionic surfactants. Niosomes may be unilamellar or multilamellar depending on the method used to prepare them. In recent years, niosome have been extensively studied for their potential to serve as carrier for delivery of drugs, antigen, hormone and other bioactive agents, the basic aim in developing delivery system is controlling the release of drugs from the carrier system, in order to achieve an extended uptake in the body, encapsulation of a drug in vesicular structure can be predicted to prolong the existence of the drug in the systemic circulation and thus enhance penetration into target tissue and reduce toxicity (3).

Niosomes similar to liposomes are biodegradable, biocompatible and non immunogenic in nature and exhibit flexibility in their structural characterization (4). In addition, handling and storage of noisome require no special conditions (5, 6). Niosomes are now widely studied as an alternative to liposomes because they alleviate the disadvantages associated with liposome such as chemical instability, variable purity of phospholipids and high cost (7). However, even though niosomes exhibit good chemical stability during storage, there may be problems of physical instability in niosome dispersions. Like liposomes, aqueous suspensions of niosomes may exhibit aggregation, fusion, leaking of entrapped drugs, or hydrolysis of encapsulated drugs, thus limiting the shelf life of the dispersion. A dry product, which could be hydrated immediately before use, would avoid many of the problems associated with aqueous niosome dispersions and problems of physical stability (aggregation, fusion, leaking). The additional convenience of the transportation, distribution, storage, and dosing would make 'dry niosomes' a promising industrial product. In the work reported here, we describe the preparation of dry niosomes, 'proniosomes'. Proniosomes are dry product which could be hydrated immediately before use(8-10). They are water-soluble carrier particles that are coated with surfactant and can be hydrated to form niosomal dispersion immediately before use on brief agitation in hot aqueous media (11).

Acyclovir, 9-(2-hydroxy) ethoxymethylguanine, is used as an antiviral agent and is especially active against herpes simplex virus and zoster herpes. Acyclovir is administered intravenously, locally, and perorally. For peroral administration, acyclovir is

produced as tablets (200 mg) that are recommended to be taken five times per day. It has been noted that not more than 20% of the acyclovir is absorbed. The daily therapeutic dose is attained by taking 400-mg tablets 12 times or 800-mg tablets 5 times. According to the FDA, the time for reaching the maximum concentration of acyclovir in blood plasma is 1.5 – 1.75 h. The biological availability of acyclovir is only 10 – 20% and decreases with increasing dose. The half-elimination period for constant kidney function is 2.5 – 3.3 h. Blood plasma proteins bind 9 – 33% of acyclovir. Acyclovir is eliminated from healthy individuals primarily with the urine, the effective absorption zone of acyclovir is the duodenum and, possibly, the upper section of the small intestine adjoining it (12).

The objective of the present study was to prepare and characterize proniosomes of acyclovir, the encapsulation of acyclovir in that vesicular structure may be expected to enhance the oral absorption and prolong the existence of the drug in systemic circulation of the drug due to the slow release of the encapsulated drug and consequently improve oral bioavailability.

2. Materials And Methods

Materials

Acyclovir was kindly supplied by Pharo Pharm Company(Alexandria, Egypt), Span 60 (sorbitan monostearate), was purchased from Sigma Chemicals Corporation, (Steinheim, Switzerland). Cholesterol 99% extra pure, Fine-Chem. Ltd. (Mumbai, India). Maltodextrin (Maltrin QDTM M500), Grain Processing Corporation, (NY, USA). Sorbitol, potassium dihydrogen phosphate, and disodium hydrogen phosphate were purchased from BDH Laboratory (BDH Chemicals Ltd, Poole, UK).All chemicals were of analytical grade.

Preparation of Acyclovir Proniosomes

Desirable characteristics of the selected carrier that could be used in the preparation of proniosomes were presented by Payne *et al*. (13, 14). These include; safety and non-toxicity, free flowability, poor solubility in the loaded mixture solution and good water solubility for ease of hydration. Examples of carriers include, sorbitol, maltodextrin, glucose monohydrate, lactose monohydrate and spray dried lactose (15).

Three different methods were adopted for the preparation of Proniosomes:

Slow spray-coating method (SSM):

Proniosomes were prepared by sequential spraying method (10, 16), where cholesterol and Span 60 in different molar ratios were dissolved in 10 ml chloroform–ethanol mixture (1:1v/v) containing 100 mg of acyclovir. The mixture was introduced into a 100-ml rounded bottom flask containing 0.5 gm sorbitol powder by sequential spraying of aliquots onto the surface of sorbitol powder. The flask was attached to a rotary evaporator to remove the solvent at 100 rpm, a temperature of 60–70° C. This process was repeated until all surfactant solution had been applied. Evaporation was continued until the content in the flask had become completely dry and freely flowing product. This dry product, referred to as proniosomes, was stored in a tightly closed container and was used for preparation of proniosome derived niosomes and for further evaluation.

Slurry method (SM):

For ease of preparation, a 250 µmol stock solution of Span-60 and cholesterol mixture was prepared in chloroform: ethanol mixture (1:1). The required volume of Span-60 and cholesterol stock solution and drug dissolved in chloroform: ethanol (1:1) solution was added to a 100 ml round bottom flask containing the maltodextrin carrier. Additional chloroform: ethanol solution was added to form slurry in the case of lower surfactant loading. The flask was attached to a rotary evaporator to remove solvent at 60 rpm, a temperature of 45 ºC ± 2, and a reduced pressure of 600 mmHg until the mass in the flask had become a dry, free flowing product. These materials were further dried overnight in a desiccator at room temperature (17). The time required to produce proniosomes is independent of the ratio of surfactant solution to carrier material and appears to be scalable (8, 18, 19).

Coacervation phase separation method (CM):

The method of preparation of proniosomes is based on the simple idea that the mixture of surfactant/alcohol/aqueous phase can be used to form the concentrated proniosomal gel, which can be spontaneously converted to stable niosomal dispersion by dilution with excess aqueous phase. This technique involves the principle of Coacervation-phase separation (20). Precisely weighed amounts of Span 60, cholesterol and drug are taken in a clean and dry wide mouthed glass vial of 10 ml capacity and ethanol (2.5 ml) was added to it. All the ingredients were mixed well with a glass rod; the open end of the glass bottle was covered with a lid to prevent the loss of solvent from it and warmed over water bath at 60-70°C for about 5 min until the surfactant mixture was dissolved completely. Then the aqueous phase (phosphate buffer pH 6.8)was added and warmed on a water bath till a clear solution was formed which was then converted into proniosomal gel on cooling (7, 21).

A total of 12 Acyclovir proniosomal formulations were prepared differing from each other in surfactant cholesterol composition using three

different methods as shown in Table 1. Each formulation was prepared in duplicate.

Preparation of niosomes from proniosomes by hydration and separation of free unentrapped Acyclovir from hydrated niosomes:

Prepared proniosome powder was weighed and filled in 20 ml screw cap vials. 10 ml phosphate buffer (pH 6.8) at 80°C (21-22) was added and the vials capped then attached to a vortex mixer and agitated for 2 minutes to get niosomal dispersion. The resulting niosomal dispersion was used for the determination of the entrapment efficiency, particle size analysis, and morphological studies.

The Acyclovir containing niosomes were separated from free Acyclovir by ultracentrifugation at 20.000 rpm for one hour at 0◦C .The supernatant was removed and the formed niosomal pellets were resuspended in phosphate buffer (pH 6.8) for 10 minutes and centrifuged again. This washing procedure was repeated two times to ensure that the free drug was no longer present in the voids between the niosomes.

Determination of Acyclovir Entrapment Efficiency in Niosomes:

Free acyclovir was separated from niosome entrapped Acyclovir by centrifugation. A 1-ml aliquot of niosome dispersion was centrifuged at 20,000 rpm at 0°C for 1 h. The supernatant was separated, and the niosomal residue was resuspended in phosphate buffer (pH 6.8) and centrifuged again. This washing procedure was repeated two times to ensure that the free drug was no longer present in the voids between the niosomes. The collected supernatant fractions were diluted to 10 ml with phosphate buffer (pH 6.8) and were used for determination of the free Acyclovir spectrophotometrically at 252 nm (23) as this wavelength represents the maximum absorbance of acyclovir in phosphate buffer (pH 6.8).

Acyclovir concentration was expressed as % entrapment efficiency which can be defined as the percent fraction of the total input drug encapsulated in the surfactant bilayers and/or aqueous compartments in the niosomes and obtained by subtracting the amount of free drug from the total drug incorporated in 1 ml niosomal dispersion (22, 24) according to the equation:

Entrapment efficiency % = [(Ct – Cr) / Ct] X100

Where Ct is the concentration of total Acyclovir and Cr is the concentration of free acyclovir.

Physicochemical characterization of proniosome powder:

Measurement of angles of repose

The angle of repose of dry proniosome powder was measured by a funnel method (10, 24). Briefly, the carrier powder or proniosome powder was poured into a funnel that was fixed at a position so that the 13 mm outlet orifice of the funnel is 10 cm above a level black surface. The powder flowed down from the funnel to form a cone on the surface, and the angle of repose was then calculated by measuring the height of the cone and the diameter of its base.

Infrared spectroscopy

IR spectroscopy was conducted using a Perkin Elmer spectrum RXIFT-IR system and the spectrum was recorded in the wavelength region of 4000–500cm^{-1}. The procedure consisted of dispersing a sample (acyclovir, Span 60, cholesterol, sorbitol, maltodextrin, and two selected proniosome powder formulations) in KBr and compressing into discs by applying a pressure of 5 ton for 5 min in a hydraulic press. The pellet was placed in the light path and the spectrum was recorded.

Differential Scanning Calorimetry (DSC):

DSC was performed to study the thermal behavior of drug alone, Span 60, cholesterol, sorbitol, maltodextrin, and two selected proniosome powder formulations. The instrument comprised of calorimeter (DSC 60), flow controller (FCL 60), thermal analyzer (TA 60) and operating software (TA 60). The samples were heated in sealed ceramic pans under nitrogen flow (25 ml/min) at a scanning rate of 10 ◦C/min from 24◦C ±1 to 300 ◦C. Empty ceramic pan was used as a reference.

***In Vitro* Characterization of Proniosomes derived niosomes:**

Microscopical and Electron microscope Examination

The vesicle formation by the particular procedure was confirmed by optical microscopy of 1000 x resolution. The niosome suspension was placed over a glass slide and fixed over by drying at room temperature, the dry thin film of niosome suspension observed for the formation of vesicles. Photomicrographs of the preparation were also obtained from the microscope by using a digital camera (25)

Selected Acyclovir niosomal dispersions were examined by transmission electron microscopy. Negatively stained samples were prepared by placing a drop of niosomal dispersion on carbon coated grid. After waiting for 2 min, to allow adsorption of the niosomes on the carbon film, the excess liquid was drawn off with a filter paper. A drop of 1% uranyl acetate aqueous solution was placed on the grid. The excess was removed with distilled water and the samples were examined by transmission electron microscopy at different magnification powers (20.000-50.000).

Particle Size analysis

A small aliquot of freshly prepared proniosomes derived niosomes was used to characterize the

particle size and size distribution, using a laser diffraction particle size analyzer operated at a wavelength 780 nm and at a measuring range of 0.04 -500µm. Results were recorded as median vesicle size and cumulative percentage frequency under size (26).

***In-vitro* release studies:**

The dissolution behavior of acyclovir proniosomes was compared with pure acyclovir powder. The dissolution studies were performed according to the US Pharmacopoeia (USP) type II dissolution apparatus (paddle method).

Samples (acyclovir proniosomes and pure acyclovir) corresponding to 100 mg acyclovir is placed into hard gelatin capsule. The dissolution medium was 730 ml 0.1N HCl (pH 1.2), after one hours, 270 ml of 0.2 M tribasic sodium phosphate was added to change the pH of test medium to 6.8, and the test was continued for a further 7 hours (17, 28). The stirring speed was 100 rpm, and the temperature was maintained at 37°C ± 1. The samples (5ml) were withdrawn at various time intervals (15, 30, 60, 120, 240, 360 and 480 min) using a syringe, filtered through 0.45 µm membrane filter, and analyzed by UV spectrophotometer at 255 and 252 nm in case of 0.1N HCl or phosphate buffer (pH 6.8) respectively . Withdrawn samples were compensated by fresh medium. The dissolution experiments were conducted in triplicate.

In order to understand the kinetic and mechanism of drug release, the result of in-vitro drug release study of niosomes were fitted into various kinetic equation like zero order (cumulative % release vs. time), first order (log % drug remaining vs. time), Higuchi's model (cumulative % drug release vs. square root of time).

***Ex-vivo* Intestinal Permeability Studies:**

The method employed was modified from experimental procedures well described by Ghosh *et al* (28). Guinea pigs (1-2 Kg) were killed by overdose with pentobarbitone administered by intravenous injection. To check the intraduodenal permeability, the duodenal part of the small intestine was isolated and taken for the *in vitro* diffusion study. Then this tissue was thoroughly washed with cold Ringer's solution to remove the mucous and lumen contents. Proniosomal derived niosomes or acyclovir solution (0.1%) in phosphate buffer (pH 6.8) was injected into the lumen of the duodenum using a syringe, and both ends of the intestine were tightly closed. Next, the tissue was placed in a chamber of organ bath with continuous aeration and a constant temperature of 37 °C ± 1. The receiver compartment was filled with 30 ml of phosphate-buffered saline (pH 6.8).

Samples (3 ml) were withdrawn at different time intervals and replaced by an equal volume of fresh buffer to maintain a constant volume and sink conditions throughout the study. The absorbance was measured using a UV-VIS spectrophotometer at a wavelength of 252 nm, keeping the respective blank. The percent diffusion of drug was calculated against time and plotted on a graph.

The apparent permeability coefficient (Papp) of different formulations was determined according to the following equation (29, 30):

$$Papp = \Delta Q/\Delta t\ (3600x\ Ax\ Co) \quad cm/s$$

Where, $\Delta Q/\Delta t$ is the permeation rate of Acyclovir across the intestine obtained from the slope of the straight line of intestinal permeability (ΔQ) vs. time. A is the exposed surface area (cm2) which kept constant and equal 1.5 cm2.Co is the initial concentration of drug injected into the lumen of the duodenum. It was 1.3 mg/ml in case of formula SSM II & SM II and 1 mg/ml in case of formula CM II. Results are the average of three runs.

Stability Studies:

Selected formulations (both proniosomal powder and proniosome derived niosomes) were tested for stability by storing them at 4°C ± 1 and at 25°C ± 2 (33). Formulations were assessed for vesicles size and residual drug content before and after storage for one month (32, 33).

3. Results and Discussion

Preparation of niosomes by standard methods results in a heterogeneous dispersion, prone to sedimentation or aggregation. Of greater concern, is the difficulty of completely hydrating the lipid film on the wall of the round-bottom flask. Even after 1 h of hydration at 60°C with vigorous shaking, there is sometimes surfactant residue remaining on the wall of the flasks, and additional time is required. Although the surfactant film is thin, a finite thickness and hydration initially occurs at the surface. This layer becomes quite viscous, and tends to remain adhered to the surface. As a result, full hydration of surfactant film is difficult, and the loss of some lipids in the preparation process is possible if one is not careful to agitate the flask for an adequate time. Compared with conventional niosomes, the preparation of niosome dispersions from these proniosomes is much more convenient. Apparently due to the great surface area of the lipid film that forms on the surface of carrier, the hydration of the proniosomes and the formation of the niosome dispersion are very easy (10).

***In Vitro* characterization of proniosome-derived niosomes**

Microscopical Examination:

Optical Microscopical images of some prepared formulations are illustrated in Fig.1. The resulting niosomes through different

techniques are very similar to those produced by conventional methods but the size distribution is more uniform (34).

The produced niosomes prepared from proniosomes examined by TEM were observed as spherical vesicles. The vesicles were discrete and separate with no aggregation or agglomeration, the smoothness of the proniosome surface may be due to (35):

(a) The filling effect of surfactant on the fine structures located on the surface of the carrier, where thicker layers of surfactant are expected to be deposited at points of deeper invaginations.

(b) In case of SSM and SM, dissolution of surface molecules of the carrier (particularly, thin or sharp features) in the solvent mixture onto the carrier surface. Once the solvent was evaporated, the dissolved carrier surface molecules as well as, other components in the solvent mixture, were recrystallized onto the new surface. As expected, these effects apparently removed some of the fine crystalline structures of the carrier powder, making the surface of proniosome powder appear smoother.

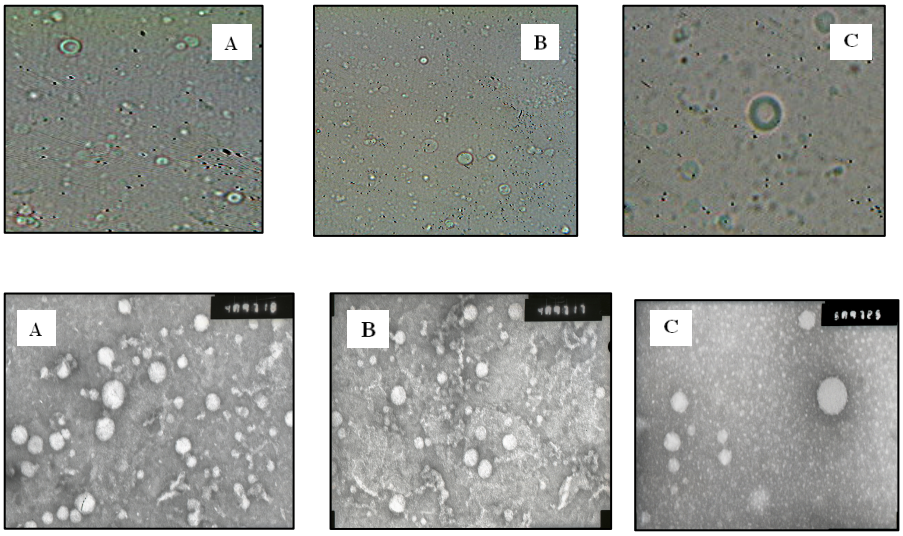

Figure 1: Optical photographs and transmission electron micrographs of Acyclovir proniosomes derived niosomes prepared by different methods; Slow spraying method (A), Slurry method (B) and Coacervation method (C).

Particle size analysis:

Mean vesicle diameter data for different Acyclovir proniosomal formulations prepared using different methods, are given in Table 1. It is clear that, higher cholesterol molar concentration resulted in increase in the mean niosome particle size. This is in agreement with the work done by Manconi *et al* (36), who observed that, as the cholesterol surfactant molar ratio decreased in niosomal formulations, reduction in size range is more evident. . Structure features of cholesterol and its lipid solubility are the reason for its influence on the particle size, i.e., it filled in empty spaces among the surfactant molecules and thus increased the particle size of the prepared vesicles (37). Formulations SSM IV, SM IV and CM IV containing no cholesterol have the smallest particle size.

Table 1: Comparative Average Vesicle Size and Percentage of Entrapment Efficiency of Different Acyclovir Niosomes Formulations

Formulation code	Composition Ratio(µmol)*	Mean vesicle size (µm)			Percentage of entrapment efficiency**		
		(SSM)	(SM)	(CM)	Slow spraying method (SSM)	Slurry method (SM)	Coacervation method (CM)
I	100:50	0.54	0.63	0.74	40.65 ±0.87	44.65±0.75	46.22±0.34
II	75:75	0.81	0.92	0.79	51.38±1.04	53.89±1.08	57.64±0.69
III	50:100	0.89	1.06	0.88	37.87±0.86	38.96±0.34	40.12±0.65
IV	150:0	0.25	0.21	0.63	29.75±0.54	30.85±0.23	32.76±0.45

*The composition ratio indicates the ratio of surfactant: cholesterol. **Values represent mean ± SD (n=3).

Entrapment Efficiency

The tendency of a drug to interact by various forces, such as polar and non polar forces, and/or electrostatic interactions with the bilayer, determines whether it would be incorporated into the aqueous compartments, into the bilayer structures or whether it would be firmly associated with the polar head groups of the bilayer, via electrostatic interaction (38). The entrapment was expressed as a percentage of the total amount of acyclovir used in preparation of the proniosomes. Among different types of surfactants, Span 60 which has high phase transition temperature, was selected as it was expected to show highest entrapment in niosomes prepared by different methods as reported in the literature (16, 32). Both Span and cholesterol have hydroxyl groups. Acyclovir possesses NH2 group, so good entrapment could be explained by hydrogen bond formation.

Entrapment efficiencies of niosomes prepared by different methods using different surfactant/cholesterol ratios are presented in Table I. Through the results obtained, it can be deduced that the increased entrapment might be the result of partly uniform vesicle size and due to well-packed bimolecular film formation; the larger vesicle size may also contribute to the higher entrapment efficiency. The data indicate an increase in entrapment efficiency on increasing total lipid concentration; higher lipid concentration might have resulted in higher encapsulation volume and thus, increases of entrapment efficiency (39). This was also similar to the results obtained by Sammour (38) using different surfactants.

It was also observed that very high cholesterol content (SSM III, SM III and CM III) had a lowering effect on drug entrapment in the vesicles (37.87%, 38.96%and 40.12%) respectively. This could be due to the fact that cholesterol beyond a certain level starts disrupting the regular bilayered structure leading to loss of drug entrapment (17, 22). Extra cholesterol is unfavorable ,it implies that equal molarity of non ionic surfactant and cholesterol can make the membrane compact and well organized (33). In addition, absence of cholesterol was found to cause low entrapment efficiency as seen in formula SSM IV, SM IV and CM IV.

Characterization of proniosome powder:

Angle of repose

Angle of repose of proniosome powders; SSMII and SM II were 36.66 and 29.37 and that of the pure carrier sorbitol and maltodextrin were 42.19 and 45.19 respectively. These results showed that the angle of repose of dry proniosome powder is smaller than that of the corresponding carrier. As a consequence the flowability of proniosome dry powder is equal to or better than that of carrier powder, so further processing of proniosome powder as beads, tablets, or capsules is possible (24).

Infrared spectroscopy

The infrared spectra for the drug, carrier, cholesterol, Span 60 and proniosome formulations SSM II, SM II are shown in Fig 2. The main absorption bands of Acyclovir are broad band at 3500 cm-1 due to (NH, OH), C = O stretching at 1600 cm-1, CH- aliphatic stretching at 2950 cm-1 and CH- aromatic stretching at 3050 cm^{-1}.The characteristic peaks of pure acyclovir have appeared in proniosome formulation, without any markable change in their position, indicating no chemical interaction between drug and carriers.

Differential Scanning Calorimetry (DSC):

Fig 3 show the differential scanning calorimetry of Acyclovir, Span 60, cholesterol, sorbitol, maltodextrin and two selected proniosomal formulations
(SSM II & SM II). There are two main peaks for Acyclovir, the first is endothermic peak starting at 68.33^{o}C and ending at 90.25^{o}C, with peak maximum at 84.14 ^{o}C which is related to water (41). The second peak starting at 250.63^{o}C and ending at 257.63^{o}C, with peak maximum at 252.49^{o}C, due to melting of Acyclovir (41).

The characteristic peak of ACV fusion appeared in both proniosomal formulations (SSM II and SM II) which did not reveal any obvious interaction. This was in accordance with the results obtained from IR studies, as mentioned before.

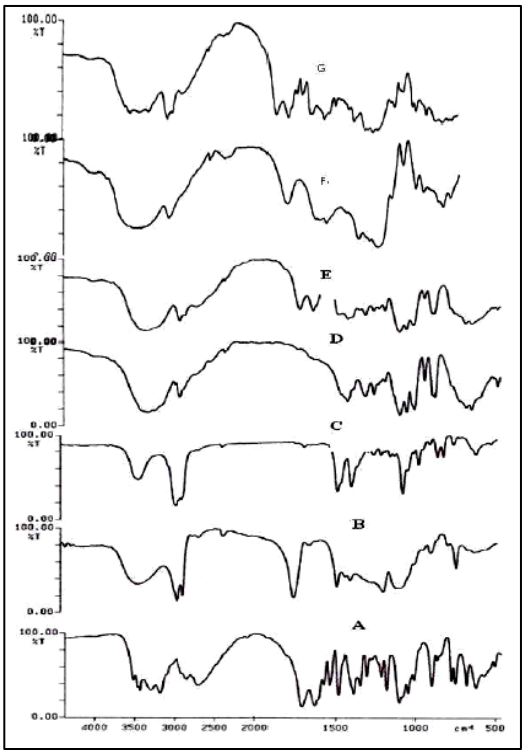

Figure 2: IR spectra of pure acyclovir (A), Span 60 (B), cholesterol(C), Sorbitol (D), formula SSM II (E), maltodextrin (F) and formula SM II (G).

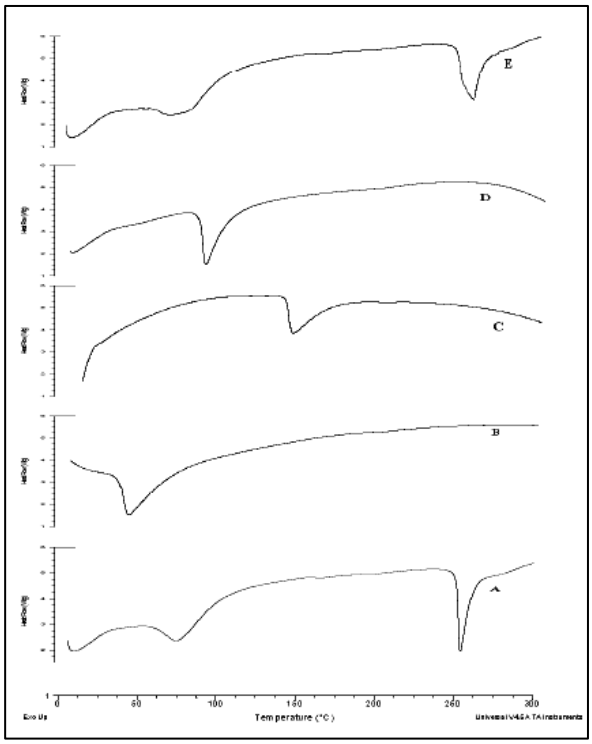

Figure 3: DSC thermogram of pure acyclovir (A), Span 60(B), cholesterol(C), sorbitol (D), formula SSM II (E), maltodextrin (F) and formula SM II (G).

Release studies:

Pure acyclovir exhibited high and fast release. It showed 72.8%, 77.8% and 89.9% release after 15, 30 and 45 min respectively and that was in agreement with the pharmacopeial requirements for dissolution of acyclovir capsules(42), which state that not less than 75% of the labeled amount of acyclovir should be dissolved in 45 minutes.

The dissolution profiles of acyclovir from proniosome containing capsules prepared by different methods are shown in Fig 4. The results pointed to an initial fast release of acyclovir from proniosomes followed by relatively slow release up to 8 hours. This first fast release may be due to the un-entrapped amount of drug then the slow release was due to the entrapped drug from proniosome-derived niosomes. As a general pattern, there was a delay in amount released with increasing cholesterol levels .A possible explanation of these findings is related to the ability of cholesterol to abolish the gel to liquid phase transition of niosomal systems and thus improve the encapsulation of hydrophilic drugs. Moreover, it decreases the niosomal membrane fluidity and enhances its rigidity by condensing the packing of surfactants in the bilayers membranes (32, 35, 43, 44).

Concerning formulations SSM IV, SM IV and CM IV which contain no cholesterol, they showed higher initial and cumulative amount released compared to other formulations, This could be related to lower amount drug entrapped (Table 1) coupled with the effect of cholesterol absence. Cholesterol induces membrane instability and increases drug leakage(17).

The correlation coefficient (r^2) was determined in each case, from which mechanism of release was predicted. Based on correlation coefficient, the results of kinetic analysis showed that the best fit was achieved with Higuchi model for the prepared formulations, which indicated that drug release mechanism from proniosome derived niosomes was diffusion. Similar results were observed in the literature (16, 37).

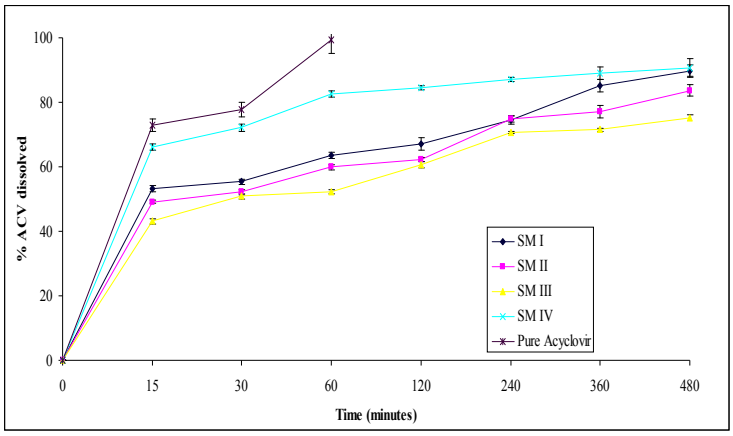

Figure 4: Release profiles of ACV proniosomes prepared by SSM**.**

Ex-vivo Intestinal Permeability Study:

Proniosome formulations (SSM II, SM II and CM II) were selected for *in vitro* permeation study based on entrapment efficiency as they showed the highest entrapment efficiency within the prepared formulations with a good release profile. In vitro intestinal permeability data are shown in Fig 5.

The drug diffused at a faster rate from the proniosome formulations than from drug solution. The total percentage diffusion was much higher for the proniosome formulations than for ACV solution. For instance, after 6 hours of diffusion, 75.89%, 79.81% and 69.11% of the drug was diffused from formula SSM II, SM II and CM II respectively, as compared with 50.61% diffused from drug solution.

Proniosomes should be hydrated to form niosomal vesicles before the drug is released and permeates across the duodenum. Several mechanisms could explain the ability of niosomes to modulate drug transfer across duodenum including ;adsorption and fusion of niosomes onto the surface of duodenum would facilitate drug permeation, the vesicles may act as penetration enhancers to reduce the barrier properties of the absorption site, and the lipid bilayers of niosomes may act as a rate-limiting membrane barrier for drugs (45). Also fusion of niosome vesicles to the surface of duodenum may result in higher flux of the drug due to direct transfer of drug from vesicles to the absorption site (46).

The apparent permeability coefficient (Papp) could be used as a parameter for the permeation

.There was a direct proportionality between the % total cumulative amounts permeated at the end of the permeation experiment and the apparent permeability coefficient values as seen in Table (2).

According to the equation

Papp= ΔQ/Δt (3600x Ax Co)

Papp depends on the exposed surface area (which was held constant) and the initial concentration of drug injected into the lumen of the duodenum. It was 1.3 mg/ml in case of formula SSM II & SM II and 1 mg/ml in case of formula CM II.

Relative Papp values were also calculated by dividing the values of the apparent permeability coefficient by Papp of reference (0.1 % drug solution in phosphate buffer pH 6.8).The relative Papp values would reflect how many times the permeability of the proniosomal formulations increased or decreased compared to the reference.

Table 2: Comparative Intestinal Permeation of Different Acyclovir Proniosomal Formulations.

Formulations	Intestinal permeability (%total cumulative amount permeated)	Apparent permeability coefficient (Papp).10^6 cm / sec	Relative Papp values (to Papp of solution)
ACV Solution	50.61	14.42	1
SSM II	75.89	22.924	1.5896
SM II	79.81	24.042	1.5896
CM II	69.11	20.97	1.4538

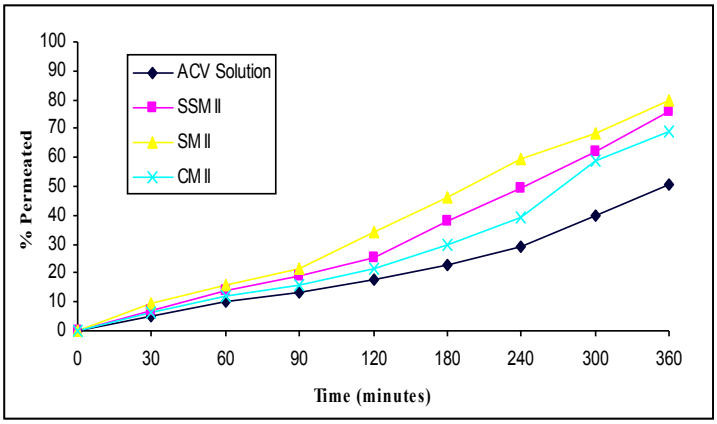

Figure 5: Comparative Ex-vivo diffusion profile of acyclovir through guinea pig

Stability Studies

In the present study, the stability of the vesicles was determined by measuring the vesicle size, and residual drug content before and after 30 days at 4°C ± 1 and 25°C ± 2 for both immediately reconstituted proniosome powder and proniosome derived niosomes for some selected formulations.

The increase in vesicle size was higher in formulations stored at 25°C ± 2 than at 4°C ±1 in case of proniosome derived niosomes indicating that prepared formulation was relatively stable at 4±1°C, as compared to 25°C ± 2.On the other hand, no difference between vesicle sizes in formulations stored at 25°C ± 2 in case of immediately reconstituted proniosome powder compared to initial vesicle size before storage, indicating the high stability of dry powder form compared to niosomal dispersion (Table 3).

Stability was also evaluated in terms of percent residual drug remaining in the vesicle, considering initial drug content as 100%. There was a marked reduction in the residual drug content when proniosome derived niosomes were stored at 25°C ± 2 rather than at 4°C ± 1; while immediately reconstituted proniosome powder showed nearly 100% residual drug content, this might be attributed to the rigidization of the vesicles at low temperature that reduced the permeability of the drug through the membrane (31, 32). On the contrary, there was no change in residual drug content in case of immediately reconstituted proniosome powder stored at 25°C ± 2 compared to initial drug content before storage, indicating again the high stability of dry powder form compared to niosomal dispersion (Table 3). Stability studies suggest that proniosomes offer a more stable system that could minimize the problems

associated with conventionally prepared niosomes like degradation by hydrolysis, or oxidation, sedimentation, aggregation and fusion during storage (35).

Table 3: Effect of Storage on Mean Vesicle Size and Residual Drug Content.

Codes	Initial vesicle size (μm)	Mean vesicle size (μm)			Percentage of residual drug		
		Proniosome derived niosomes		Reconstituted proniosome powder At 25±2°C	Proniosome derived niosomes		Reconstituted proniosome powder At 25±2°C
		At 4±1°C	At 25±2°C		At 4±1°C	At 25±2°C	
SSM II	0.81	0.91	1.06	0.81	93.2±1.56	63.2 ±2.53	100
SM II	0.92	0.98	1.13	0.91	96.12±2.48	72.5±1.52	100
CM II*	0.79	0.87	1.01	0.78	90.3±1.68	54.8 ±1.68	97.8*

* CM produces white creamy proniosomal gel from which niosomal dispersion is reconstituted.

Conclusion

Proniosomes are promising drug carriers for the future with greater physical and chemical stability and potentially scalable for commercial viability. The delivery system holds promise for the effective drug delivery of amphiphilic drugs. Proniosomes in dry powder form makes the possibility of convenient unit dosing as the proniosome powder can further be processed to make beads, tablets or capsules. The findings of the studies on proniosomes till date open the door for the future use of different carrier materials with biocompatibility and suitability for the preparation of proniosomes. However, future experiments should explore the suitability of proniosomes with more drugs having defined drawbacks for improved and effective intended therapy. Studies should be explored to assess the ability of different carrier materials to formulate proniosomes and the ability of proniosomes to deliver the drugs meant for administration through various routes.

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