Research Article

Formulation, Characterization and Invitro Evaluation of Flurbiprofen Niosomes

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Abstract
Niosomes or non-ionic surfactants vesicles are microscopic lamellar structures formed on the admixture of a non-ionic surfactant, cholesterol and flurbiprofen with subsequent hydration in aqueous media. The delivery of drugs by "vesicular drug delivery system" such as niosomes provides several important advantages over conventional drug therapy. The main objective of this study was to design suitable niosome-encapsulated drug delivery for anti-inflammatory drugs like flurbiprofen and evaluate the vesicle size, encapsulation efficiency and in vitro release of prepared niosomes. Non-ionic surfactants used were span ٢٠, ٤٠, Brij ٥٢ and cholesterol was used in different molar ratios. The niosomes prepared using lipid film hydration method. The higher entrapment efficiency was observed with niosomes prepared from span ٤٠ and cholesterol in a ١:١ molar ratio.
Keywords: Formulation, Niosome, Cholesterol, flurbiprofen, Span.

Introduction
Topical drug delivery is the most common way for treatment of a number of anterior segment diseases of the eye due to ease of use, patient compliance and safety[١,٢].

However, ocular drug delivery has a considerable bioavailability problem as less than ١٠٪ of the instilled drug is absorbed due to the ocular defense. Rapid elimination through nasolacrimal drainage and dilution by tear turn over. Consequently, frequent instillation of the topical ocular formulations is needed in order to achieve the desired therapeutic effects[٣,٤]. Thus, efficient drug delivery for treatment of various ocular disorders is considered a challenge[٥].

Niosomes are considered as colloidal particles in which a concentric bilayer of amphiphilic molecules surrounds an aqueous compartment. When used for drug delivery, hydrophobic drugs are retained within the lipid bilayer while the hydrophilic drugs are encapsulated in the interior aqueous compartment (Figure ١).Niosomes or non-ionic surfactants vesicles are microscopic lamellar structures formed on the admixture of a non-ionic surfactant, cholesterol and flurbiprofen with subsequent hydration in aqueous media [٦].

Materials and methods
Materials
Flurbiprofen was obtained as a gift sample from Egyptian International Pharmaceutical Indusries Co. (EIPICO), (Egypt.). Spans (٢٠, ٤٠, and Brij ٥٢) were purchased from Fluka Chemical Co. (India). Cholesterol, chloroform and methanol were purchased from ADWIC, El-Nasr Pharmaceutical Co., (Egypt). Solvents and other reagents were of analytical grade.

Methods
Preparation of flurbiprofen niosomes by lipid film hydration method:
Different niosomal formulations were prepared by lipid film hydration technique. accurately weighted quantities of surfactant (either span ٢٠,٤٠ or Brij ٥٢) and cholesterol in different molar ratios ١:١, ٩:١, ٨:٢, ٧:٣, ٦:٤ were dissolved in ١٥ml of a chloroform / methanol mixture (٢:١, v/v) in a round bottom flask. The solvent mixture was evaporated in a rotary flash evaporator and the flask rotated at ١٠٠ rpm until a smooth, dry lipid film was obtained. The film was hydrated with ١٠ ml of PBS

٦.٨ containing for ٦٠ minute at ٦٠˚C with gentle shaking on a water bath[٨].

Characterization of flurbiprofen Niosomes: Entrapment efficiency percentage (EE%):

This is determined by measuring the difference between the total and the unentrapped amounts of the drug. The proportion of encapsulated FBP was obtained by ultra-centrifugating ١ ml of the niosomal suspension at ١٥,٠٠٠ rpm for ١ hour using a cooling centrifuge at ٤ °C. The supernatant was used to determine the amount of the unentrapped drug spectrophotometrically at ٢٤٦ nm after appropriate dilution. The EE was calculated using the following equation [٩-١١].

$$EE\ \% = \frac{\text{Total amount of drug - unentrapped drug amount}}{\text{Total amount of drug}} X١٠٠$$

Morphology:

The morphology and the surface characteristics of niosomes were examined using scanning electron microscopy (SEM).

Particle size analysis:

The particle size and size distribution of the niosomes were measured using laser light diffraction particle size-analyzer. For a typical experiment, the samples were properly diluted with PBS and measured at ٢٥ ^{o}C and analyzed. The measurements were done in triplicate and the average values were used.

In-vitro drug release study

The release of FBP from niosomes was determined using the membrane diffusion technique. An accurately measured volume of FBP niosome formulations, equivalent to ٠.٠٣mg/ml transferred to a glass cylinder (١٠ cm length and ٢.٥ cm diameter) that was sealed at its lower end with presoaked cellulose membrane fitted by elastic bands. The glass tube was suspended in a shaking water bath containing ٥٠ ml phosphate buffer (pH ٦.٨). The glass tube was allowed to rotate at a constant speed (٥٠ rpm) the phosphate buffer was maintained at a temperature of ٣٧±٠.٥°C. [٩].

Results And Discussion

Entrapment efficiency of flurbiprofen niosomes

To obtain the highest encapsulation efficiency, several factors, including the inclusion of cholesterol and the structure of the surfactant were investigated and optimized.

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Effect of cholesterol content

These results can be explained by the fact that an increase in cholesterol content resulted in an increase of micro viscosity of the membrane indicating more rigidity of the bilayers. Cholesterol has the ability to cement the leaking space in the bilayer membranes. It is obvious from table (٢) that further increase of cholesterol content, reaching a highest ١:١ molar ratio of surfactant: cholesterol for niosomes composed of span ٤٠ was found to here increase entrapment efficiency.

Effect of the surfactant

Data in table (٢) reveals that the entrapment efficiencies for niosomes prepared using span ٤٠ were superior to those prepared using span ٢٠. This can be explained by many facts: (a) the hydration temperature used to make niosomes should usually be above the gel to liquid phase transition temperature of the system that results in niosomes that are less leaky and have high entrapment efficiency. Span ٤٠ has higher phase transition temperature (٥٠˚C) as compared to Span ٢٠ and hence high entrapment efficiency. (b) The length of alkyl chain of surfactant has a prominent effect on permeability of prepared niosomes as length of surfactant increases entrapment efficiency also increases and as length decreases entrapment efficiency also decreases. Hence long chain surfactant results in high entrapment. Thus span ٤٠ has a longer saturated alkyl chain (C١٤) compared to span ٢٠ (C١٠), so it produces niosomes with higher entrapment efficiency. On the other hand all formulations prepared from Brij ٥٢ show phase separation, so these samples excluded from any farther analysis.

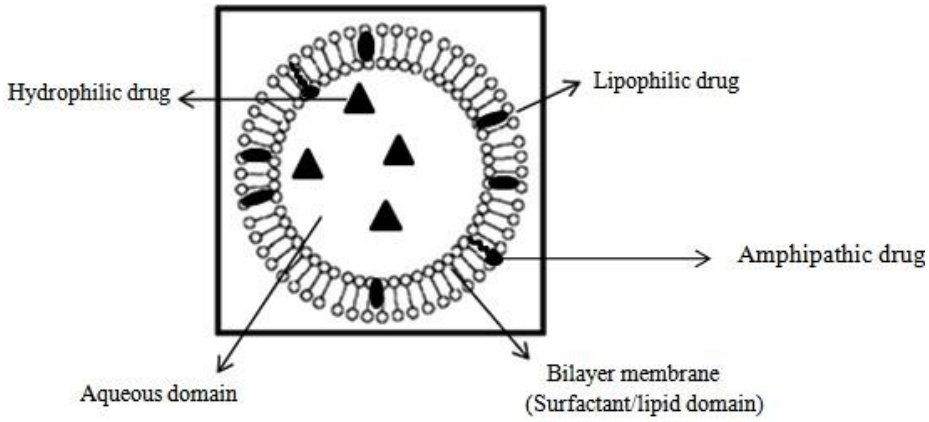

Figure ١: Locations of entrapped hydrophilic, lipophilic and amphipathic drugs in niosomes [٧].

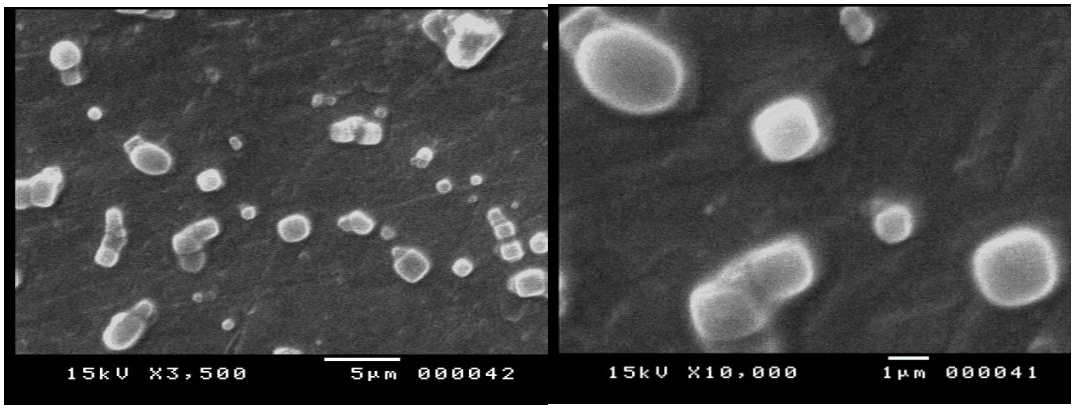

Figure ١: SEM images of F-١ niosomes at magnification powers: x (٣٥٠٠) &Figure٣: SEM images of F-١ niosomes at magnification powers: x (١٠٠٠٠).

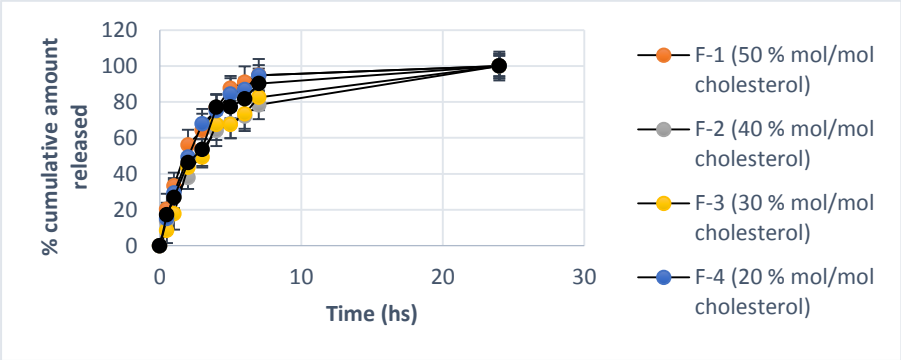

Figure ٤: Effect of cholesterol concentration on niosomes prepared from Span ٤٠ on the release of flurbiprofen from different formulae at ٣٧° C.

Table ١: Niosomes formulation composition

Formulae Code	Surfactant used	Surfactant: Cho١esterol Ratio (µmol)
F١	Span ٤٠	١:١
F٢	Span ٤٠	٦:٤
F٣	Span ٤٠	٧:٣
F٤	Span ٤٠	٨:٢
F٥	Span ٤٠	٩:١
F٦	Span ٢٠	١:١
F٧	Brij ٥٢	١:١
F٨	Brij ٥٢	٦:٤
F٩	Brij ٥٢	٧:٣

Table ٢: Entrapment efficiency of flurbiprofen niosomes

Formula code	Drug Entrapment efficiency EE (%)
F١	٦٦.٥ ±١.٥٦
F٢	٦٥ ±٤.٨
F٣	٦٤.٦ ±١.٧
F٤	٦٣.٤ ±١.٢٣
F٥	٦١.٢ ±١.٥٢
F٦	٦٥ ±٠.٩

Table ٣: Particle size distribution of different formulae of flurbiprofen loaded niosomes.

Formula code	Mean Particle size (µm)
F١	٢.٠٥
F٢	٠.٩٨١
F٣	١.٠٣
F٤	١.٤٤
F٥	٣.٦٥
F٦	٠.٧١

Morphology:
The microstructure and lamillarity of the prepared niosomes were studied using scanning electron microscope (SEM). The micrographs captured revealed multilamellar spherical-shaped niosomes that exist in disperse and aggregate collections. Images of the formulae prepared using span ٤٠ and span٢٠ are shown in Figures (٢, ٣) that revealed a complete spherical shape of the prepared niosomes with smooth surface.

Particle size analysis
Table ٣ shows that the niosomes prepared using span ٤٠ have larger size than those prepared using span ٢٠. This might be attributed to the longer saturated alkyl chain of Span ٤٠ compared to span ٢٠ as it was reported that the longer the alkyl chains the larger the vesicles size. This would account for the higher entrapment efficiencies of niosomes prepared from span ٤٠[١٢].

In Vitro Release of flurbiprofen from Niosomes
Results of in vitro study on the release of flurbiprofen from niosomal vesicles prepared using span ٤٠ and cholesterol in molar ratios ١:١, ٩:١, ٨:٢, ٧:٣, ٦:٤ are shown in figure (٤). From the results it is obvious that as the molar conc. of cholesterol increased from ٩:١ to ٦:٤, causes marked reduction in the efflux of the drug, which was in accordance with its membrane stabilizing ability. Cholesterol is known to abolish the gel to liquid phase transition of niosomal systems, resulting in niosomes that are less leaky increasing the cholesterol beyond a certain level ٦:٤ starts disrupting the bilayered structure leading to loss of drug entrapment levels in this case.

Conclusion
The main objective of this study was to design suitable niosome-encapsulated drug delivery for anti-inflammatory drugs like flurbiprofen to study the in vitro behavior of the prepared system and to investigate the niosome encapsulated drug for its activity. Finding of all this investigation conclusively demonstrate prolongation of drug release at a constant and controlled rate, after encapsulation of flurbiprofen. It shows that niosomal drug delivery system may be a promising carrier for the novel drug delivery system.

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