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NIOSOMAL CARRIER PREPARATION OF PREDNISOLONE FORMULATION AND IN-VITRO EVALUATION

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ABSTRACT

Niosomes are microscopic lamellar vesicles build from mixture of nonionic surfactant, cholesterol and phosphate buffer with subsequent hydration in aqueous media, capable of entrapping hydrophilic and hydrophobic molecules. The aim of this study is to formulate prednisolone loaded niosomes. Prednisolone is a synthetic corticosteroid commonly used as an anti-inflammatory agent and immunosuppressant. Niosomes are formulated by ether injection method and evaluated for following parameters (a) vesicular diameter (b) Entrapment efficiency (c) in-vitro drug release. Non-ionic surfactants used were span 80 and cholesterol used in 1:2:1 molar ratio. The niosomes prepared were large unilamellar vesicles (LUVS) with higher entrapment efficiency of 69.5 %. The *in-vitro* diffusion study suggests that higher entrapment efficiency comparatively causes slow release. The results conclusively demonstrates prolongation of drug release at a constant and controlled rate into systematic formulation, have reduced protein–binding, cheap, chemically stable, thus overcoming systemic side effects, reducing administered dose, improving therapeutic efficacy and patient compliance.

KEYWORDS: **Prednisolone, Ether Injection technique, Niosomes, and Cholesterol**.

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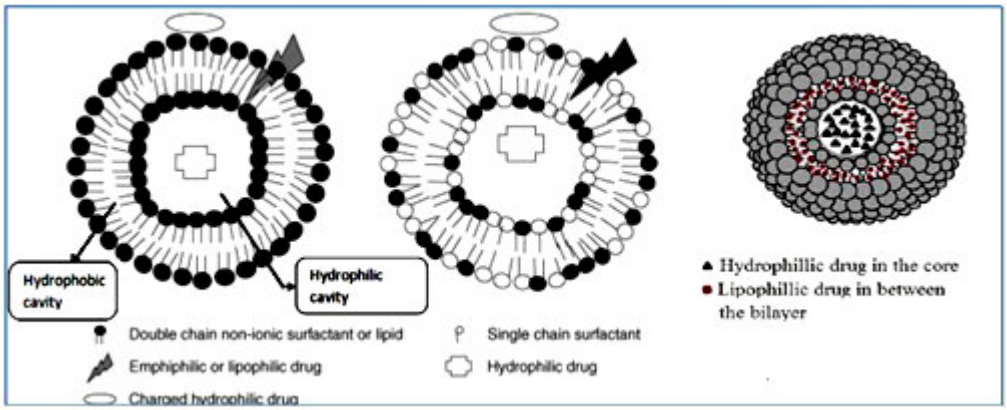
1. INTRODUCTION

Niosomes are synthetic lamellar structures that are microscopic vesicles in size consisting of an aqueous core enclosed in a bilayer consisting of cholesterol and one or more nonionic surfactants with subsequent hydration in aqueous media.[1,2, 3] The surfactant molecules tend to orient themselves in such a way that the hydrophilic ends of the non-ionic surfactant point outwards, while the hydrophobic ends face each other to form the bilayer .[4, 5, 6, 7] They are made of biocompatible, biodegradable, non-toxic, non-immunogenic and non-carcinogenic agents which form closed spherical structures (self-assembly vesicles) upon hydration. With high resistance to hydrolytic degradation, niosomes are capable of entrapping many kinds of soluble drugs while exhibiting greater vesicle stability and longer shelf life. Controlled release drug products are often formulated to permit the establishment and maintenance of any concentration at target site for longer intervals of time. One such technique of drug targeting is niosomes. They behave *in-vivo* like liposomes prolonging the circulation of entrapped drug and altering its organ distribution. Niosomal drug delivery has been studied using various methods of administration including intramuscular, intravenous, oral, and transdermal. In addition, as drug delivery vesicles, niosomes have been shown to enhance absorption of some drugs across cell membranes, to localize in targeted organs and tissues and to elude the reticuloendothelial system. Niosomes have been used for application such as anticancer, anti-tubercular, anti-leishmanial, anti-inflammatory, hormonal drugs and oral vaccine.[4, 7, 9] Prednisolone (11β)-11, 17, 21-trihydroxypregna-1, 4-diene-3, 20-dione is a steroidal drug with predominant glucocorticoid and low mineral corticoid activity. It is mainly used for the treatment of a wide range of inflammatory and autoimmune diseases such as asthma, multiple sclerosis, rheumatoid arthritis, autoimmune hepatitis[10] etc. Prednisolone is also known as 'disease modifying antiarthritic drugs' because of its anti-inflammatory action by inhibiting gene transcription for COX-2, cytokines, cell adhesion molecules, and inducible NO synthetase. When steroidal anti-inflammatory drugs such as prednisolone are given orally results in systemic side effects like bone loss, increased susceptibility to infection, osteoporosis, peptic ulcers and buffalo hump. Parental route of administration results in rapid clearance rate of drug which ultimately compels invasive and frequent administration of drug.[10]The currently available methods, have a dose limiting therapeutic index with compromised safety implications. Attempts will be made in developing and characterizing a specific drug delivery system targeting drugs to blood circulation which in turn increase drug efficacy with minimum toxicity.

1.1. Advantages of Niosomes

The vesicle suspension is water- based vehicle. This offers high patient compliance in comparison with oily dosage forms, [2, 4, 9] they possess an infrastructure consisting of hydrophilic, amphiphilic and lipophilic moieties together and as a result can accommodate drug molecules with a wide range of solubility's,[2 ,7, 9] the characteristics of the vesicle formulation are variable and controllable. Altering vesicle composition, size, lamellarity, tapped volume, surface charge and concentration can control the vesicle characteristics; [2, 4, 7, 8, 9, 12] the vesicles may act as a depot, releasing the drug in a controlled manner. [2, 4, 6, 9, 12]Other advantages of niosomes include that they are osmotically active and stable, as well as they increase the stability of entrapped drug,[2, 4, 6, 7, 9, 13] handling and storage of surfactants requires no special conditions,[2, 4, 6, 7, 9,11,12, 13,14] they improve oral bioavailability of poorly absorbed drugs and enhance skin penetration of drugs,[2, 4, 6, 7, 9,11,12] they can be made to reach the site of action by oral, parenteral as well as topical routes,[2, 4, 6, 16] the surfactants are biodegradable, biocompatible and non-immunogenic,[2, 4, 6, 8, 11, 15] they improve the therapeutic performance of the drug molecules by delayed clearance from the circulation, protecting the drug from biological environment and restricting effects to target cells [7, 8, 11, 12] and niosomal dispersion in an aqueous phase can be emulsified in a non-aqueous phase to regulate the delivery rate of drug and administer normal vesicle in external non-aqueous phase.[2, 7, 12,15]

Figure 1
Schematic representation of a niosome & 3-dimension structure [1, 2, 3]



2. MATERIALS AND METHODS

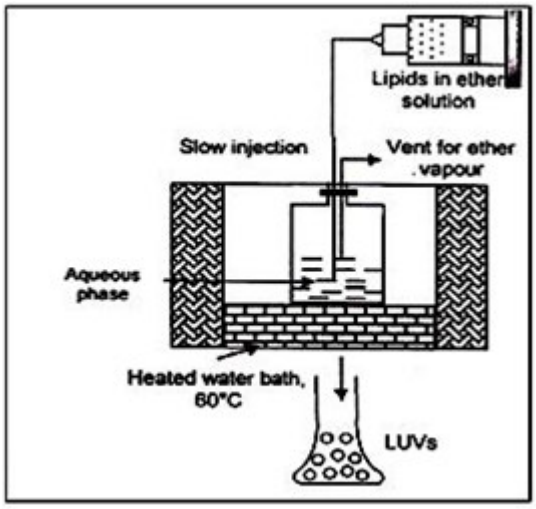
***2.1. Materials*:** Prednisolone was obtained as a gift sample from AL-Jazerah pharma, Span 80, and Cholesterol was obtained from Loba Chemie Pvt. Ltd., Mumbai. Diethyl ether obtained from Scharlau Ltd., sodium phosphate monobasic and sodium phosphate dibasic was supplied from Acros organic Ltd., Mumbai.

2.2. Method for preparation
Ether Injection method
Prednisolone loaded niosomes using drug: Span 80: cholesterol as an enhancer of niosomal membrane rigidity were prepared using 1:2:1 design as in table 1.[15, 16] Niosomes were prepared by weighing calculated amount of span 80 and cholesterol in a 50 ml beaker. The mixture was dissolved in 10 ml of diethyl ether and the solution was drawn into a 14 gauge needle.[1, 4, 6] Then the solution was slowly injected at a rate of 1 ml/min into a beaker containing calculated amount of drug in 20 ml of phosphate buffer pH 7.4 and was stirred simultaneously with a magnetic stirrer.[1, 13, 15] The temperature maintained during the injection was 60ºC. Figure 2. [1, 2, 4]

Table 1
Composition of Prednisolone niosomes

Formulation code	Drug: Surfactant: Cholesterol (ratio)	Drug (mg)	Span 80 (mg)	Cholesterol (mg)
1	1:2:1	100	200	100

Figure 2
Ether Injection method [2, 4]



2.3. Separation of non-entrapped Drug
The removal of non-entrapped solute from the vesicles is accomplished by:

Gel Filtration
The unentrapped drug is removed by gel filtration of niosomal dispersion [2, 7] through a Sephadex-G-60 column and elution with phosphate buffered saline.[12]

a. Gel Chromatography Column Preparation
Weight out 5.0000 g of Sephadex-G-60 (Sigma, G-50-80) into a 500 ml beaker. Fill up the beaker with PBS to 3 times the original volume of Sephadex-G-60. Allow 1 hour for a complete absorption of Sephadex-G-60. Pipette Sephadex-G-60 solution into a gel chromatography column slowly. There should be no air bubbles present during packing process. Allow Sephadex-G-60 to settle down gravitationally and repack the column again until desired column height.

b. Gel Exclusion Chromatography Separation
Pipette 1 ml of the sample and place that into the top of the column and let the sample settle down for 1 minute. Allow the sample to flow through the column.
Note: there should be clear color separation. Collect the sample and wrap each flask with paraffin paper for further analysis.

3. Evaluation of Niosomal dispersion

3.1. Organoleptic properties
Niosomal dispersion was characterized for appearance color, odor, and pH.

3.2. Size Analysis
By optical microscopy
A drop of noisome suspension was placed on a glass slide and it was diluted. A cover slip was placed over the diluted niosome suspension and evaluated the average vesicle size and shapes by an ordinary optical microscope [4, 8] using (40 x magnifications) a precalibrated ocular eye piece micrometer.[1] Particle size analysis was carried out using about 200 niosomes were measured individually, average was taken and their size distribution range and mean diameter were calculated. Further microphotographs of optimized niosomes were taken by using 9 megapixel Sony DSC-W110 digital camera.

3.3. Percentage drug entrapment (PDE)
Free prednisolone was separated from entrapped

prednisolone in niosomes by ultracentrifugation [1, 3, 7, 15] where 1ml niosomal dispersion was frozen for 24 h at -20ºC in Eppendorf tubes. The sample were removed from the freezer let to thaw at room temperature then centrifuge at 13000 rpm for 40 min at 4ºC using an ultracentrifuge model LE-400R with rotor type 70Ti (T, Californhermo scientific, Germany) to separate supernatant and pellets at the bottom then 0.1 ml of Supernatant containing free prednisolone was collected and analyzed by UV – visible spectrophotometer at λ_{max} 243 nm. Percent drug entrapment (PDE) was calculated by using following formula, the results of PDE are listed in table 3. [2, 4, 6, 8, 14]

$$PDE = \frac{\text{Total amount of drug} - \text{Amount of drug detected in supernatant layer}}{\text{Total amount of drug}} x\ 100$$

3.4. Encapsulated Drug Measurement

a. Calibration curve of prednisolone

The calibration curve of prednisolone was prepared in phosphate butter pH 7.4. For determination of absorption maxima, a solution of 10 microgram/ml of prednisolone in PBS was prepared and then absorbance is determined from 200 nm to 400 nm Using U.V. spectrophotometer. Then 100 mg of prednisolone was weighted accurately and dissolved in 10ml of methanol (10% v/v) and 90 ml of phosphate buffer pH 7.4 (PBS). Volume was making up 100 ml by PBS. Then 1 ml of this solution was diluted to 10 ml by 10% methanolic buffer pH 7.4 to produce 100 µg/ml stock solution. From this stock solution, aliquots of 2.5 ml, 5 ml, 7.5 ml, and 10 ml were taken and diluted suitably by 10% v/v methanolic solution of phosphate buffer pH 7.4. The calibration curve was plotted b/w concentration and absorbance.

3.5. In-vitro release of prednisolone from niosomes

The release of prednisolone from niosomes was determined using the membrane diffusion technique, [2,4,6,8] 1 ml of niosomal suspension was placed in a diffusion cell (glass tube) of diameter 2.5 cm, the lower open end of the glass tube was covered with soaked cellulose membrane. This cell then suspended in the beaker containing PBS pH 7.4 (100 ml). This was constantly stirred at speed 50 rpm at 37 ±1°C on a magnetic stirrer with a thermostat. Aliquots were withdrawn at hourly intervals and replaced simultaneously with equal volume of fresh PBS. The prednisolone concentration in the samples was analyzed by spectrophotometer, as mentioned earlier. The obtained data were analyzed to determine the amount and mechanism of drug release. [1, 8, 15]

4. RESULTS AND DISCUSSION

4.1. Calibration curve of prednisolone

Calibration curve of prednisolone in 10% methanolic solution of phosphate buffer pH 7.4 at 243 nm and the absorbance values of different serial dilution concentrations of prednisolone in phosphate buffer methanolic solution (pH 7.4) are shown in table (4). Calibration curve in figure (4) of different serial dilution concentrations of drug in 10% methanolic solution of phosphate buffer (pH 7.4) vs. absorbance was showed the highest linearity and beer's lay obeyed in the concentration range of 10-30 µg/ml on the basis of coefficient of regression (R^2); data had correlation coefficient (R^2) values close to one, i.e., (R^2=0.997052).

4.2. Characterization of Prednisolone Niosomes

4.2.1. Organoleptic properties

The niosomal dispersion was off-white in color, odorless, and fluid in nature. It was stable and did not show sedimentation. PH was found to be in the range of 4.7-5.2.

4.2.2. Photo microscopy

The photo micrographs of Prednisolone niosomes prepared by ether injection method are shown in figure (3). They reveal that the niosomes were spherical in shape and exist in disperse and aggregate collections.

4.2.3. Determination of vesicle size

The mean particle diameters of niosomes, composed of span 80 with cholesterol as shown in table (2) showed that unilamellar niosomes spherical in nature are large in size with mean particle size of 3.1247 ± 1.54 due to slow injection rate of 1 ml/min of span 80 with cholesterol solution into a beaker containing calculated amount of drug. In addition to that span 80 has a longer saturated alkyl chain that generally give larger vesicles. Also the large mean size of the niosome increased with decrease in the HLB value of span 80 because surface free energy decreases on increasing hydrophobicity of surfactant.[10]

4.2.4. Entrapment Efficiency

After the separation of un-entrapped drug by ultracentrifuge, the entrapment efficiency of the formulation was studied. The various factors like lipid concentration, drug: lipid ratio, cholesterol content will change the entrapment efficiency. The lipophilicity of surfactant also influences the entrapment of drug. The formulation with cholesterol and span 80 in the ratio of 1:2 showed entrapment efficiency of 69.5%, were found to be optimum for loading maximum amount of Prednisolone in niosomal formulation. 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. This would account for the higher entrapment efficiencies with span 80 niosomes.The entrapment efficiency with span 80 niosomes is found high also due to low HLB of span 80 that is 4.3. The lower the HLB of the surfactant the higher was the drug entrapment efficiency.

4.2.5. In-vitro drug release study

In- vitro study was carried out for niosomes formulation prepared from span 80 showed a good percentage of drug release for about 4 hrs. The results were

represented in table 5 and graph 5 below. The release rate is assumed to be based on lipophilicity of the surfactant. The Span 80, being relatively lipophilic, impedes the easy permeation to the aqueous phase. At the end of 4 hour, 15.355% Prednisolone was released from span 80 niosomes. From the obtained results it was observed that drug release was prolonged for 4 hours by incorporating drug in niosomes. This can be explained by the fact that niosomes exhibit an alkyl chain length dependent release. The higher the chain length, the lower the release rate of drug from formulations. It is to be noted that the in-vitro release results are consistent with those of entrapment efficiency, as the unilamellar niosomes composed of span 80 and cholesterol (2:1) molar ratio with the high entrapment efficiency (69.5%) showed good drug release percent after 4 h (T_4h=15.355%). Thus results of *in vitro* release study of Prednisolone from niosomal vesicles indicate that by encapsulation of drug into niosomes, it is possible to sustain the release of the drug for a longer duration of time as the niosomes act as reservoir system for continuous delivery of drug.

Table 2
Vesicle size of Prednisolone Niosomes

Formulation code	Mean particle size µm ± SD
1	3.1247 ± 1.54

(Mean ± S.D., n=200)

Figure 3
Photomicrographs of prednisolone loaded niosomes composed of span 80 and Cholesterol in 2:1 molar ratio by Ether injection method.

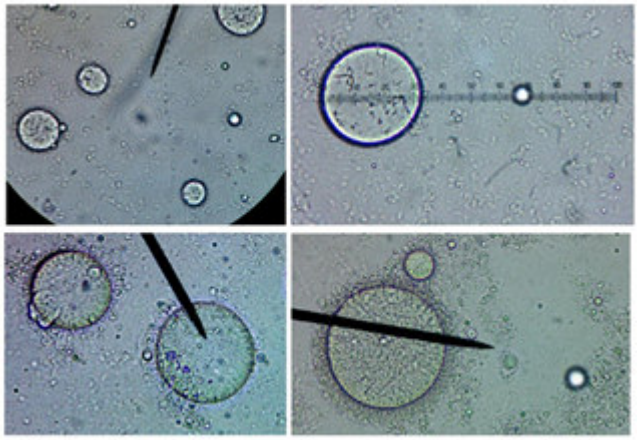


Table 3
Encapsulating efficiency of prednisolone niosomes

Niosomal Formulation Code	Ether Injection Method (EIM)		
	Absorbance	Concentration (µg/ml)	Drug Entrapment (%)
1	2.251	9.673	69.5

Table 4
Calibration curve of prednisolone

Concentration	Absorbance
0	0
2.5	0.632
5	1.213
7.5	1.822
10	2.431

Table 5
In vitro release profile of prednisolone niosomes (The percentage cumulative drug release after 4 hr. from niosomal vesicles)

Time (Hrs.)	Absorbance	Concentration (µg/ml)	% cumulative drug Released
0	0	0	0
1	0.625	2.426	12.13
2	0.664	2.764	13.82
3	0.741	2.955	14.775
4	0.75	3.071	15.355

Figure 4
Calibration curve of prednisolone

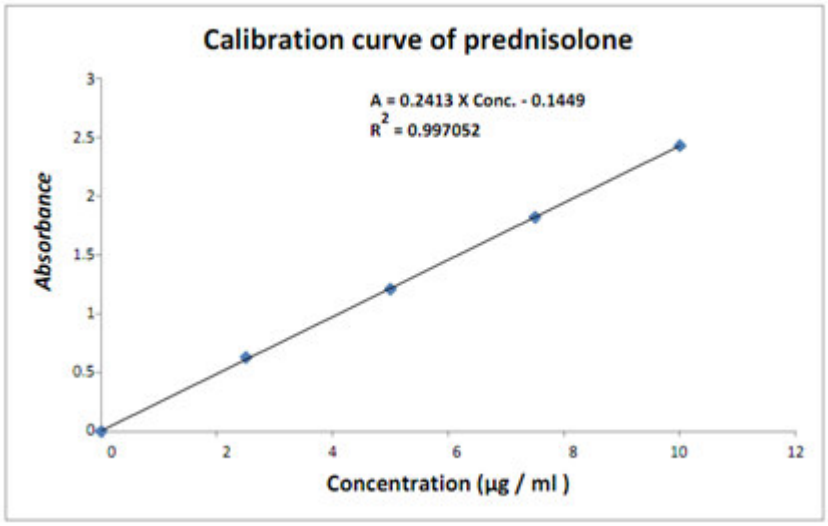
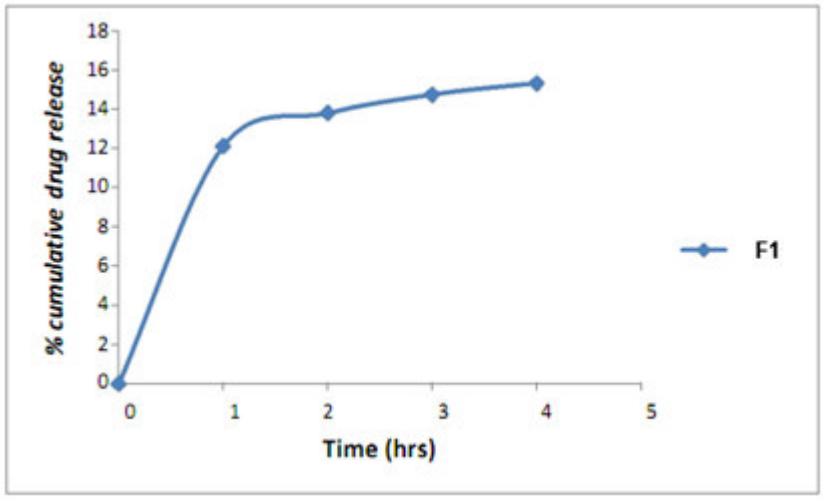


Figure 5
In-vitro release of prednisolone loaded niosomes prepared by ether injection method



5. CONCLUSION

Niosomes containing anti-inflammatory prednisolone were prepared by an ether injection technique using nonionic surfactant span 80 and cholesterol at ratio 1:2:1 showed good results. This formulation showed high entrapment efficiency, having drug release for 4 hrs. In conclusion, the niosomes formulation of prednisolone showed a promising novel drug delivery system, which had shown issues regarding patient's compliance and side-effects.

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