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Comparative evaluation of niosome formulations prepared by different techniques

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Abstract

Niosomes containing salbutamol sulphate was prepared using Span 60 as the surfactant, by employing different techniques namely, thin film hydration, hand shaking, ether injection, lipid layer hydration and trans membrane pH gradient method. The drug encapsulation efficiency varied from 62 % to 87 %. *In vitro* drug release studies was carried out and formulation exhibited retarded release for 24 h. Transmembrane pH gradient method was found to be most satisfactory which released 78.4 % of drug in 24 h. This formulation was lyophilized and characterized by infrared spectroscopy. Tissue distribution studies in albino rats and bio- availability studies in rabbits were carried out.

Keywords: Different niosomal methods, bioavailability, comparative study.

Introduction

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. Niosomes are microscopic lamellar structures formed on admixture of a nonionic surfactant, cholesterol and diethyl ether with subsequent hydration in aqueous media. They behave *in vivo* like liposomes prolonging the circulation of entrapped drug and altering its organ distribution. (Baillie et al. 1985).

The low cost, greater stability and ease of preparation of non-ionic surfactant has lead to exploitation of these compounds as alternatives to phospholipids (Schreier 1985). In recent years non-ionic surfactant vehicles received great attention as potential drug delivery systems for different routes of administration.

Antiasthmatic drugs have high potential to induce toxic side effects. Therefore it is desirable to deliver them to target tissue in the right manner at the right time. Salbutamol sulphate is a selective β_2 adrenergic agonist. The plasma half-life is 2-7 h (Goodman and Gilman 1990). So the aim of the present work is to investigate the influence of the different methods of preparation of niosomes using salbutamol sulphate as a model drug, so that greater therapeutic efficacy can be attained with controlled delivery.

Materials and Methods

Materials

The drug salbutamol sulphate was a gift sample from Jagdale Scientific Research Foundation, Bangalore. Span 60, Cholesterol and ether were purchased from loba Chemie, Mumbai .Sephadex G-50 was purchased from Aldrich Thomas Co., USA.

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All other reagents used were of analytical grade.

Preparation of Niosomes

Thin film hydration

Twelve milligram each of span 60 and cholesterol (1:1) ratio were dissolved in ether and the solvent was evaporated at room temperature, using rotary flash evaporator. 10 ml of the aqueous phase containing drug (1.2 mg/ml) was added to this at 7 ^{0}C and shaken for about 15 min resulting in good dispersion of the mixture.

Hand shaking

Twelve milligram each of span 60 and cholesterol (1:1) were dissolved in ether and solvent was evaporated at 20 ^{0}C using rotary flash evaporator. The film was hydrated by aqueous solution of drug (1:2 mg/ml) at 50 ^{0}C to 60 ^{0}Cfor 15 minutes (Baillie et al. 1985, Martin 1990).

Ether injection

Twelve milligram each of span 60 and cholesterol mixture was dissolved in 20 ml diethyl ether and injected slowly (0.25 ml/min) through 14 gauge needle into 12 ml of aqueous phase maintained at 60 ^{0}C (Baillie et al. 1986).

Lipid layer hydration method

Twelve milligram each of span 60 and cholesterol (1:1) were dissolved in chloroform and the solvent was evaporated using rotary flash evaporator. 10 ml of phosphate buffer saline PH7.4 containing drug (1.2 mg/ml) was added to the dried thin film with gentle agitation. The mixture was intermittently mixed on a vortex mixer. Sonic dispersion of the mixture was carried out at 25 ^{0}C using probe sonicator set at 200 watts for 1 minute. (Azmin et al.1985).

Transmembrane pH gradient

Twelve milligram each of span 60 and cholesterol (1:1) were dissolved in chloroform. The solvent was then evaporated under reduced pressure to get a thin film on the wall of the round bottom flask. The film was hydrated with 300 mm citric acid (PH 4) by vortex mixing. Then the product was frozen. To this niosomal suspension 5 ml aqueous solution containing 1:2 mg/ml drug was added and vortexed. The pH of the sample was raised to 7 – 7.2 with 1 ml disodium hydrogen phosphate. (pH 7.4) (Mayer 1985).

Purification

The prepared niosomes were purified to remove unentrapped drug by passing through sephadex G-50 column. The elution was carried out using phosphate buffer saline solution (pH 7.4) (Ijeoma 1998).

Vesicle characterization

Two milliliter of niosomes was taken in a small container and sonicated for 5 min using a needle probe type sonicator (Braun sonic 1410) set at 400 watts. 1 ml of the above solution was pipetted into 50 ml volumetric flask and the volume was made up with distilled water. From the stock solution 5 ml was pippeted into 25 ml volumetric flask. The colour was developed using 4 Aminophenazone and absorbance was measured at 505 mm using spectronic – 20. The entrapment efficiency was calculated.

Table 1. Entrapment efficiency of different formulations

Formulation code	Method used	Entrapment efficiency % ±SD
A	Thin film hydration	78.12±0.079
B	Hand shaking	62.50±0.064
C	Ether injection	67.70±0.078
D	Lipid film hydration	72.91±0.208
E	Transmembrane	87.51±0.239

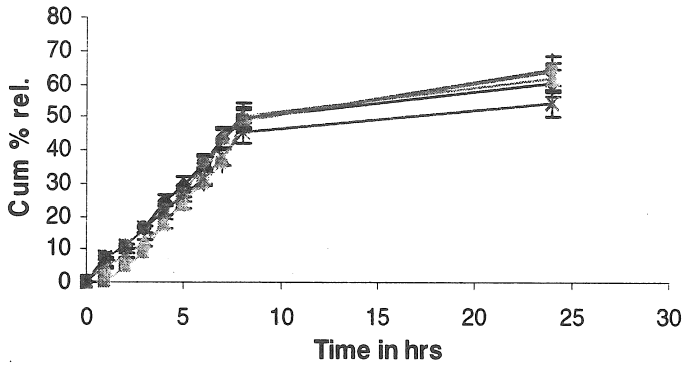
Particle size determination

The niosomes were observed and measured along an arbitrarily chosen fixed line. Optical microcopy technique was used. A total of about 100 niosomes were observed and their size coinciding with the eye piece micrometer was recorded. Most of the niosomes were found to be spherical in shape, few being with triangular and slightly elongated. The mean diameter was found to be 3.25, 5.62, 3.79, 2.53 and 3.06 μm in case of A, B, C, D and E formulations. (Martin 1985).

In vitro release studies

Sigma dialysis bag containing 2 ml of niosomes was tied at both ends and was placed in 100 ml beaker containing 50 ml of phosphate buffer saline solution of pH 7.4 maintained at 37 ^{0}C and stirred using a magnetic stirrer. At specified interval of time 5 ml of the sample solution was with drawn from the beaker and replaced with fresh buffer solution. The sample was analysed after necessary dilution and treatment with reagents. The *in vitro* release data is shown in Figure 1.

Figure 1. Comparative In vitro release profile



Key: Formulation A (♦); Formulation B (■); Formulation C (▲); Formulation D (ж)

Lyophilisation

One of the niosome formulation prepared by transmembrane pH gradient that showed maximum in vitro release was taken in a tube. 10 % w/v of mannitol was added as cryoprotective agent. The tubes were well stirred under closed condition and freezed in the cooling bath of the lyophiliser at a temperature of 40 ^{0}C and a pressure of 10^{-2} for 5-6 h. then they were lyophilized by subjecting to a high vacuum-cooling trap, where the sample was dried to powder. Then they were kept in a desiccator till use. The drug content was estimated. The IR spectra of pure drug and the lyophilized product were determined by KBr disc method.

Tissue distribution profile

Healthy albino rats weighing around 150-180 g were divided into 3 group comprising of 3 animals in each group. The rats were injected with free drug in-group 2. Group 1 was treated as control, which received free niosomes without the drug. Group 3 received the lyophilized product. The rats were sacrificed after injection of drug. Various tissues like liver, lungs, spleen, kidney and heart were removed and assayed for drug content. The tissues were washed with phosphate saline buffer pH (7.4) and homogenized. The homogenized products were centrifuged. The supernatant liquid was taken for the determination of drug content.

Table 2. Tissue distribution data for formulation E using transmembrane pH gradient method

Organ	Drug content in percentage
Liver	7.96
Kidney	5.57
Lungs	75.62
Spleen	3.98
Heart	3.98

Bioavailability

Healthy rabbits weighing 15-20 kg were used. They were divided into 4 groups of 3 animals. They were injected with free drug, empty niosome, lyophilized product and marketed drug. 1 ml of blood was withdrawn at 0,1,2,3,4,6,7 and 24 h. Heparinized blood samples were centrifuged at 5000 rpm for 10 minutes. Plasma was separated from the blood. The plasma proteins were precipitated with 0.5 ml of 5M HCL. The supernatant liquid was made up with water to 10 ml and estimated for drug content.

Table 3. Comparative Pharmacokinetics study data

Pharmacokinetic parameters	Pure drug	Conventional dosage form	Niosome formulation
Peak height max ug/ml	0.85±.oo4	.80± 0.028	0.85± 0.034
Time of peak t (max) h	3±0	4±0	6±0
Area under the curve (AUC)	3.3± 0.0029	5.17±0.056	5.36±.064
Elimination rate constant	0.3450±0.023	0.1810±0.053	0.0995±0.058
Absorption rate Constant	0.748±0.049	0.495±0.039	0.309±0.034
Biological halflife E ½	2.008±0.043	3.828±0.035	6.964±0.056
Mean absorption time (MAT) h	1.336±0.14	2.020±0.19	3.236±0.045

In vitro and *in vivo* correction was carried out by taking % drug release in vivo and % drug release in vivo. The best fitting is shown in the Figure 2.

Figure 2. Best fit for formulation E

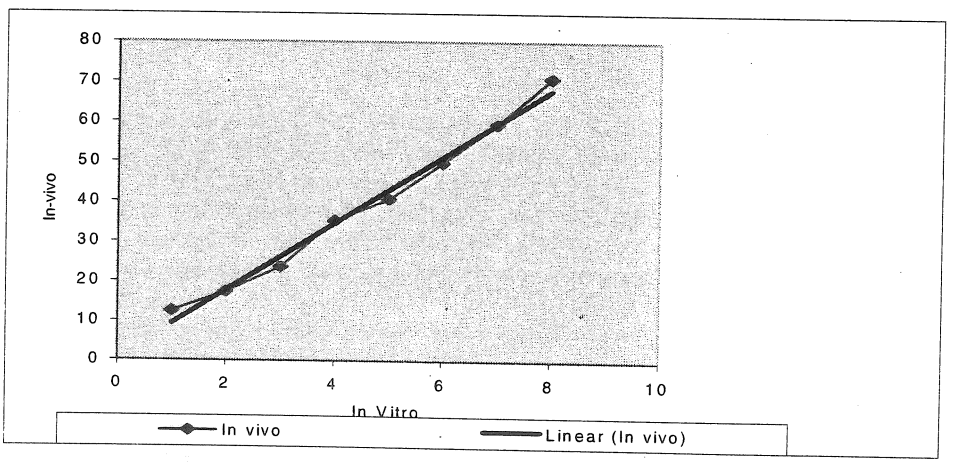


Table 4. *In vitro in vivo* correlation

In vitro	In vivo
12.5	11.25
17.26	18.75
23.57	26.25
35.11	37.5
40.47	45.0
49.64	65.25
59.33	60.0
70.97	56.25

Correlation co- efficient (r) = 0.995; Eg. For best fit time
Y=0.369+0.991 X X; $\gamma = 0.995$

Results and Discussion

Salbutamol sulphate, cholesterol and span 60 were used in equal ratios and niosomes prepared using different techniques. The drug entrapment efficiency was found to be between 62%–87%. According to figure 1, the release rate were found to be 67.07 %, 64.33 %, 62.03 %, 64.0 % and 78.47 % for A, B, C, D and E formulation respectively. *In vitro* release suggests that all the formulations exhibits retarded release for 24 h. Most of the niosomes were found to be spherical in shape, few being either triangular or slightly elongated. The mean diameter was found to be 3.25, 5.62, 3.79, 2.53 and 3.06 μm in case of A, B, C, D and E formulations.

Since niosome formulations are stable only at 4^0, the formulation E was lyophilized. 1 ml of the formulation gave 150 mg of the lyophilized product. After reconstitution of the product in saline, the niosomes were found to be spherical in shape. The IR spectra of the lyophilized product and the pure drug gave the same kind of peaks proving the intactness of the drug in the formulation. From tissue distribution data it is seen that 75 % of the formulation concentrates in the lungs that shows that targeting can be achieved.

Thus niosomes prepared by different methods have the same composition, but showed different characteristics in dimension, entrapment efficiency and release rate. Transmembrane pH gradient method was found to be most satisfactory which is simple and reproducible. In this process the presence of a net charge, whether negative or positive can increase water uptake within the double layer. Such hydration leads to an increase with respect to uncharged vesicles of loaded hydrophilic molecules that can probably be located within the bilayer as well as in the core of the aggregated structures. Thus niosomes prepared by this method helps in formulating prolonged activity dosage from with targeting. Formulation of niosome with molar ratio of 1:1 is most beneficial for the efficient encapsulation, and extra cholesterol is unfavorable. It implies that equal molarity of non ionic surfactant and CH can make the membrane compact and well organised. (Yonggmei et al., Hao 2002)

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