

Formulation and Evaluation of Paclitaxel Niosome for its Improved Anti-Cancer Activity

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Abstract

The present work was to formulate Paclitaxel niosomes and evaluate for its *in vitro* cytotoxic studies using HEP-2 and HELA-human cancer cell lines. Drug-excipients interaction study revealed that there was no interaction between the drug and excipients. Formulations made with span 60 was the most satisfactory with the entrapment 97.0%; particle size 800-850 nm; the release was found to be 95.6% after 17 h and compared the LD₅₀ with the standard formulation Taxol. Effects of volume of organic solvent, surfactant and cholesterol ratio were also studied. The stability study results revealed that the prepared formulation was stable in the stress condition.

Keywords: Paclitaxel, niosome, cancer cell, HEP-2, HELA.

Introduction

For effective chemotherapy an optimal concentration of chemotherapeutic agent must reach the affected tissue and remain there for a period of time. Since no of chemotherapeutic agents are cytotoxic, the presence of drug in the non-diseased tissue can lead to serious side effects. In the past two decades several advancements have been made. They have resulted in the development of new techniques for drug delivery (Chein 2000). Encapsulation of drug in the vesicular structure can be predicted to prolong the drug in the circulation and enhance the penetration into target tissues, and perhaps reduce toxic side effects if selective uptake has achieved (Jain et al. 2006, Shyamala and Lakshmi 2009).

These techniques are capable of controlling the rate of drug delivery, sustaining the duration of therapeutic activity and targeting the delivery of drugs to a cell or tissue. In the site targeting drug delivery system the drug molecules are circumventing the other tissues and moves towards the specific diseased site and get released. This will enhance the therapeutic effectiveness and reduces the toxicity to other healthy tissues and improve the treatment of spectrum.

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An approach has been undertaken to alter the distribution characteristics in the body by incorporating the antitumor drugs into macromolecular carriers which are expected to be concentrated at the tumor site; macromolecular carriers that have been exploited recently as liposomes or niosomes.

One such technique of drug targeting is niosomes (Azmin et al. 1985). Non ionic surfactant vesicles (niosomes) are widely studied as a novel and efficient drug delivery system, which are spherical lipid bilayers which can entrap hydrophilic solutes within the non aqueous domain and hydrophobic solutes within the lipid bilayers, able to reduce some disadvantages associated with liposomes (Attia et al. 2007). Niosomes are vesicles composed of natural phospholipids, surfactants and cholesterol and they seem to offer many advantages as drug carriers, quite stable, biodegradable, biocompatible, non-immunogenic in nature, exhibit flexibility in their structural character, no special condition is required for production and storage. Presence of non ionic surfactant with the lipid, there is better targeting of drugs to tumor, liver and brain which may increase the absorption of certain drugs (Aarti and Deepali 2001, Dufes et al. 2004, Biju et al. 2006).

Paclitaxel is a diterpenoid pseudoalkaloid, chemically 5β , 20-epoxy-1,2 α , 4, 7 β , 13 α -hexahydroxy tax-11-en-9-one 4,10-diacetate 2-benzoate 13-ester with (3R, 350-N-benzoyl 1-3-phenyllisoserine), the first of a new class of microtubule stabilized agents has been hailed by National Cancer Institute. For anti-tumor activity entire taxane molecule is required, since the ester and the tetraol formed by a low temperature cleavage of paclitaxel are found to be essentially inactive (Wiernik et al. 1987, Sonnichsen and Relling 1994, Sonnichsen et al. 1995, Walle et al. 1995, Wall and Wani 1996).

In the present investigation, our main objective was to develop paclitaxel niosomes, evaluate for its physicochemical properties and antitumor activity by using human cell lines over a prolong period of time and thereby reducing the potential dose related side effects.

Materials and Methods

Materials

Paclitaxel, Cholesterol, Span 20, 40, 60 and 80 and dicetyl phosphate were procured from Sigma Chemicals Ltd, Mumbai, India, Chloroform from Ranbaxy, Ethanol, Potassium dihydrogen phosphate, sodium chloride and sodium hydroxide were procured from Nice Chemicals, India.

Preparation of niosomes

For preparing the niosomes surfactant, cholesterol, dicetyl phosphate were dissolved in 10 ml of chloroform. The solvent was evaporated under reduced pressure leaving a thin layer of solid mixture deposited on the wall of the round bottom flask using a rotary evaporator. Paclitaxel is dissolved in 2 ml of ethanol and phosphate buffer pH 7.4 was added to the flask and heated to 50°C on the water bath and intermittently mixed on a vortex until a good dispersion of the mixture was obtained. The suspension was then sonicated to form unilamellar niosomes. The prepared niosomes were filled into a narrow ended glass tube, and the unwanted electrolytes were separated from niosomes by dialysis method. The various compositions of the formulations are shown in Table 1.

Table 1. Composition of Paclitaxel niosome

Material	F1	F2	F3	F4
Paclitaxel (mg)	4	4	4	4
Chloesterol (mg)	73.5	73.5	73.5	73.5
Dicetyl phosphate (mg)	3	3	3	3
Span 20 (mg)	73.5	-	-	-
Span 40 (mg)	-	73.5	-	-
Span 60 (mg)	-	-	73.5	-
Span 80 (mg)	-	-	-	73.5
Chloroform (ml)	10	10	10	10
Ethanol (ml)	2	2	2	2

Compatibility Studies

Excipients are integral components of pharmaceutical dosage forms. Stability of a formulation amongst other factors depends on compatibility of the drug with the excipients and it is of significance that physical and chemical interaction can affect the bioavailability and stability of the drug. DSC, FTIR and UV techniques were commonly used to investigate the compatibility between the drug and the various excipients used in the formulation.

Encapsulation efficiency

Entrapment efficiency was determined by direct method using detergents. The membranes are broken by using 1 ml of 2.5% sodium lauryl sulphate in phosphate buffer saline in 0.1 ml of niosome formulation and made up to 5 ml with phosphate buffer saline. Then the content was incubated at 37°C for 1.5 hrs to complete breakup of the niosome membrane and to release the entrapped material. The sample was filtered through a 0.25 µm millipore membrane filter. The filtrate was measured spectrophotometrically at 227 nm and the amount was determined from the standard curve. The percent drug loading for the prepared niosomes was calculated by using the following formula.

$$\% \text{ Drug Loading} = \frac{\text{Entrapped drug in (mg)}}{\text{Total added drug (mg)}} \times 100$$

Morphological characterization of niosomes

The surface morphology of niosomes was investigated using scanning electron microscopy (SEM) by mounting on stubs using double-sided adhesive tapes. The stubs were then vacuum-coated with gold-palladium alloy using coat sputter JFC 1100 (JEOL, Japan) and the microspheres were observed and examined using SEM (JEOL JSM 6308).

In vitro release studies

In vitro release studies were carried out in a dialysis tube method. One ml of niosome formulation was taken in a dialysis tube and placed in 50 ml of phosphate buffer saline pH 7.4 at 37°C. Samples were withdrawn at various time intervals and analyzed by UV spectrophotometrically at 227 nm and the amount of drug release was determined.

In vitro cytotoxicity studies (Kan et al. 1999, Simamora et al. 1998)

Cell lines and culture medium

HEP-2 and HELA cell cultures were used in the experiments (obtained from National center for cell sciences, Pune and Pasteur Institute of India, Connoor). Stock cells of HRP-2 and HELA cell lines were cultured in RPMI-1640 and DMEM supplemented with 10% sheep serum, penicillin (100 IU/ml), streptomycin (100 µg/ml) and amphotericin B (5 µg/ml) in a humidified atmosphere of 5% carbon dioxide at 37°C until confluent. The cells were dissociated with TPVG solution (0.2% trypsin, 0.05% glucose, 0.02% EDTA in phosphate buffer saline). The stock cultures were grown in 110 ml flat bottles

and all experiments were carried out in 96 well microtiter plates, where the cell population was adjusted to 10,000 cells per well.

Cytotoxic assay

The cytotoxic assay was carried out using 0.1 ml of cell suspension containing 10,000 cells. The cells were seeded in each well of a 96 well micro titer plate (Nune and Tarsons). Fresh medium containing different concentration of the control and formulated sample were added after seeding. Control cells were incubated without the drug and with DMSO (solvent). The very little percentage of DMSO present in the wells (maximal 0.2%) did not affect the experimental results. The micro titer plates were incubated at 37°C in a humidified incubator

Stability studies

The formulation which showed best *in vitro* release was selected for stability studies. The accelerated stability studies were conducted according to the ICH guidelines for a period of 6 months.

Results and Discussion

Nowadays niosomes, the non-ionic surfactant vesicles, have received widespread attention can entrap both hydrophilic and lipophilic drugs and useful for targeting the drugs for treating cancer, viral and other microbial diseases.

Niosomes were by prepared by rotary evaporation method. Compatibility studies were carried between the drug and the common excipients by DSC, FTIR and UV techniques. The DSC, FTIR and UV results revealed that there is no interaction between the drug and the excipients used in the formulation. Entrapment efficiency is a critical parameter for the niosomes and it was determined by direct method using detergent. The entrapment efficiency for found to be 97.0% and 85% for F3 and F4, whereas others formulations F1 and F2 showed around 60% of entrapment. Hence formulation F3 and F4 was taken into consideration for further studies. The particle size and shape of the formulations were analyzed by SEM and it was found to be 800-850 nm and 692-740 nm for span 60 and span 80 respectively. The scanning electron micrograph of niosomes of formulations F3 and F4 are shown in Figure 1 and 2. The SEM figures revealed that niosomes are almost spherical in shape with smooth surface.

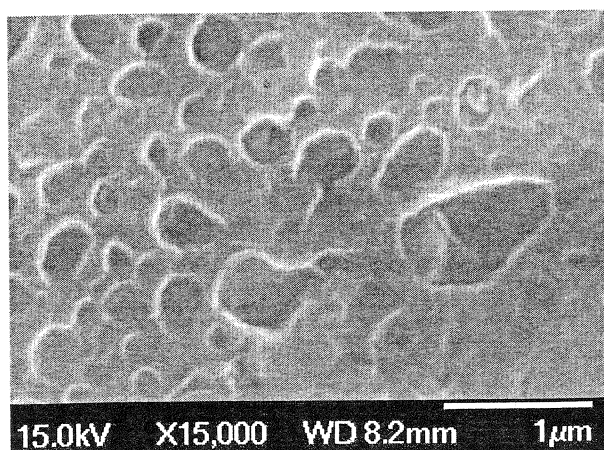


Figure 1. SEM micrograph of F3

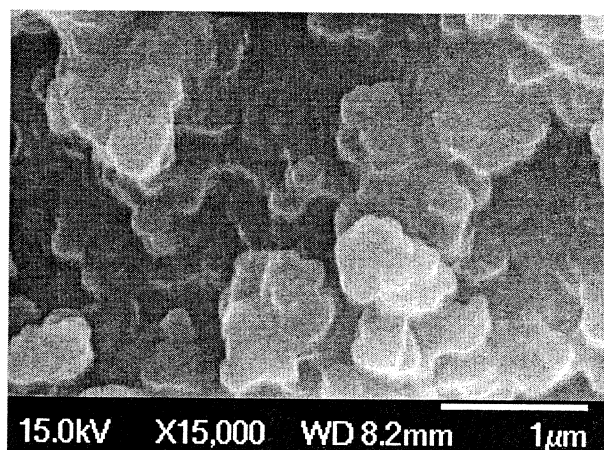


Figure 2. SEM micrograph of F4

The *in vitro* release studies were carried out by dialysis tube method using phosphate buffer pH 7.4, samples were withdrawn at specific time interval and analyzed by spectrophotometrically. The cumulative percentage release after 17 h was found to be 95.6% and 84.11% for F3 and F4 respectively. The release results are shown in Figure 3.

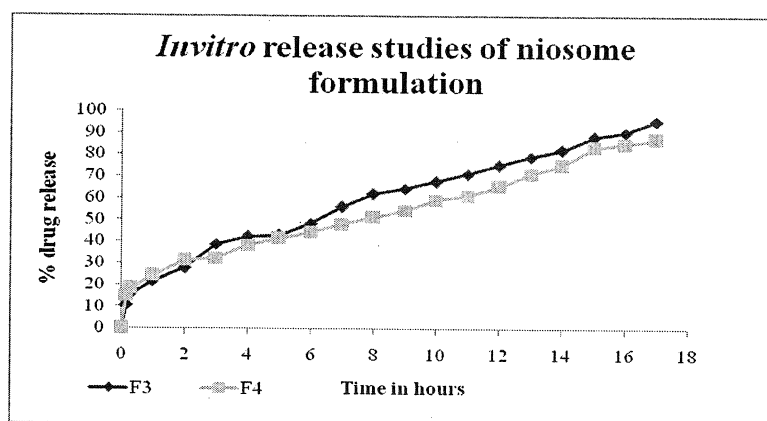


Figure 3. *In vitro* release studies of niosome formulation F3 and F4

From the above results formulation made with span 60 was taken for LD₅₀ determination. The LD₅₀ were determined by *in vitro* studies by MTT assay method [3-(4,5-dimethylthiozole-2yl)-2,5-diphenyl tetrazolium bromide] using HEP-2 and HELA cancer cell line and compared with standard formulation Taxol. The LD₅₀ results were found to be 9.87µg/ml and 8.12 µg/ml for HEP-2 cancer cell line; 6.2 µg/ml and 7.0 µg/ml for HELA cancer cell line for F3 and Taxol respectively.

Effect of surfactant and cholesterol ratio were studied, the results revealed that the surfactant and cholesterol had considerable effect in the encapsulation efficiency; however particle size distribution of niosome was more dependent on the volume of the organic solvent rather than the surfactant cholesterol.

Stability studies were carried out at 4°C and 25°C and tested for its properties and *in vitro* release studies, the stability study results revealed that the prepared formulation is stable in the stress condition.

Conclusion

Niosome were prepared by thin film evaporation method. The formulation prepared with span 60 as surfactant showed high entrapment efficiency when compared to others. The prepared niosome were almost spherical in shape with smooth surface. The observed results are promising in terms of its stability, extended release profile and comparable LD₅₀ values with the standard formulation of Taxol performed by MTT assay technique. Further studies can be done on humans to ascertain its suitability for the effective treatment.

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