

International Journal of Research in Pharmaceutical and Nano Sciences

Journal homepage: www.ijrpns.com



THE EFFECT OF SPAN 60 IN THE FORMULATION OF NIOSOMES CONTAINING FLUTAMIDE

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ABSTRACT

The current investigation were aimed to develop sustain release formulation of Flutamide niosomes in order to provide better therapeutic effect. Flutamide niosomes was prepared by thin film hydration method using drug, span 60 and cholesterol in different ratios. The formulations were optimized from the above method with respect to vesicle shape, entrapment efficiency, drug content, compatibility studies and *in vitro* drug release. The FT-IR spectra shows the drug and excipients were compatible. The *in vitro* release studies indicates that all the formulation exhibits retarded release for 24 hrs and its release mechanism was followed by Higuchi order kinetics.

KEYWORDS

Niosomes, Cholesterol, Span 60 and Non-ionic surfactant.

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INTRODUCTION

Non-ionic surfactant vesicles are now widely studied as an alternative to liposomes. Niosomes are essentially non-ionic surfactant based multilamellar or unilamellar vesicles in which an aqueous solution of solute is entirely enclosed by a membrane resulted from the organization of surfactant macromolecules as bilayers. Similar to liposomes niosomes are formed on hydration of non-ionic surfactant film, which eventually hydrates imbibing or encapsulating the hydrating aqueous solution¹.

Niosomes are microscopic lamellar structure of size range between 10-1000nm and consists of biodegradable, non-immunogenic and biocompatible surfactants. The niosomes are amphiphilic in nature, which allows entrapment of hydrophilic drug in the core cavity and hydrophobic drugs in the non-polar region present within the bilayer hence both hydrophilic and hydrophobic drugs can be incorporated into niosomes².

Flutamide is a non-steroidal anti androgenic drug used for the treatment of prostate cancer. This drug has quit extensive first pass metabolism, shorter elimination half-life and poor bioavailability, which reduces testosterone only when administration on a continuous basis. High dose of Flutamide produces hepatotoxicity.

Flutamide is effective in prostate cancer treatment only when its desired concentration in blood is maintained in longer duration. Flutamide liposome's using egg phosphatidylcholine and cholesterol retained in plasma whereas free drug disappeared from blood circulation after 24hrs. But main disadvantage was long term stability problem in case of liposomes. Niosomes may deliver the Flutamide for longer duration in blood and will expect a better stability when compared to other particulate dosage form³.

MATERIAL AND METHOD

Flutamide was obtained from Cipla Ltd Mumbai, India. Span 60, cholesterol, chloroform and methanol was obtained from S.D.Fine chemicals.

Preparation of niosomes⁴

Flutamide niosomes was prepared by using thin film hydration method using vacuum rotary evaporator. Accurately weighed quantity of cholesterol and surfactant were dissolved in chloroform-methanol mixture (1:1 V/V) in 100 ml round bottom flask. The weighed quantity of drug is added to the solvent mixture. The organic solvents were slowly evaporated under reduced pressure using rotary evaporator, at 58-60 °C at 150 rpm for 30-40 min. After evaporation of the organic solvent a thin film formed on the inner wall of the rotating flask is then hydrated with 10 ml phosphate buffer of pH 7.4 for

1 hr at 58-60 °C at rpm 150. The resultant dispersion was then cool in an ice bath and sonicated for 3 min at 150 V to get the niosomal formulation. The prepared niosomal formulations were stored in a refrigerator for the further studies.

EVALUATION OF NIOSOMES⁵⁻⁷

Vesicle size analysis

Vesicle size analysis was carried out using an optical microscope with a calibrated eyepiece micrometer. About 300 niosomes were measured individually, average was taken and their size distribution range and mean diameter were calculated.

Surface morphology

The samples are dried thoroughly in vacuum desiccator before mounting on brass specimen studies, using double sided adhesive tape. Gold-palladium alloy of 120 °A Knees was coated on the sample sputter coating unit (Model E5 100 Polaron U.K) in Argon at ambient of 8-10 °C with plasma voltage about 20mA. The sputtering was done for nearly 5 minutes to obtain uniform coating on the sample to enable good quality SEM images.

Zeta potential

Zeta potential of the niosomes was measured by PALS zeta analyser. The zeta analyser mainly consists of laser which is used to provide a light source to illuminate the particles within the sample. For zeta potential measurements this light splits to provide an incident and reference beam. The incident laser beam passes through the centre of the sample cell, and the scattered light at an angle of about 13° is detected. when an electric field is applied to the cell, any particles moving through the measurement volume will cause the intensity of light detected to fluctuate with a frequency proportional to the particle speed and this information is passed to the digital signal processor and then to a computer. Zeta analyser software produces a frequency spectrum from which the electrophoretic mobility hence the zeta potentials calculated.

Entrapment efficiency

For the determination of entrapment efficiency (EE %), the unentrapped drug is first separated using centrifugation method. The resulting solution is then separated and supernatant liquid is collected. 1 ml of supernatant was taken and diluted with phosphate buffer upto 10 ml and absorbance was recorded at 303 nm using UV spectrophotometer.

The % entrapment was determined by following formula:

$\% EE = \frac{\text{Amount of entrapped drug}}{\text{Total amount added}} \times 100$

Drug content

Flutamide content in niosomes was assayed by an UV spectrophotometric method. Niosomes containing equivalent to 10 mg of drug were dissolved in a 10 ml of methanol. After suitable dilution absorbance was measured by UV spectrophotometer against blank at λ_{max} 303 nm and drug content was calculated.

In Vitro drug release studies: Diffusion study

The *in vitro* release of Flutamide niosomes were studied by open ended cylinder method. The diffusion cell apparatus consist of a glass tube with an inner diameter of 2.5cm, open at both ends. One end of the tube tied with dialysis membrane which serves as a donar compartment. The niosomes equivalent to 10mg of drug was taken in this compartment and placed in a beaker containing 75ml of phosphate buffer pH 7.4 stirred at a moderate speed maintaining the room temperature. Periodically 5ml of samples were withdrawn same volume of medium was replaced. The samples were assayed by UV Spectrophotometer at 303nm using phosphate buffer pH 7.4 as blank and cumulative % of drug released was calculated and plotted against time (t).

Stability studies

To confirm the stability of niosomal formulation, Intermediate stability testing studies was performed for 6 months. The optimized formulation was kept at 30 ± 2 °C and $65 \pm 5\%$ RH and 4 ± 2 °C in stability chamber. Drug particle size, entrapment and drug release were fixed as physical parameters for stability testing.

RESULTS AND DISCUSSION

Incompatibility studies

Drug-excipients compatibility studies were carried out using FT-IR. The characteristic peak obtained of pure drug (Flutamide) and their physical mixture (1:1:1) was shown in Figure No.1 and 2. The characteristics of pure drug are also found in physical mixture indicating there was no significant interaction between the drug and excipient.

Drug content and entrapment efficiency

F1-F4 formulation having maximum amount of drug content because of increased entrapment efficiency. The entrapment efficiency of niosomes prepared at varied concentration of surfactants: Flutamide: cholesterol concentration is shown in Table No.2. If increasing the surfactant ratio from F1-F4 the entrapment efficiency increases.

Vesicle size analysis

The sizes of the niosomes are measured using an optical microscope with calibrated eyepiece micrometer. From each batch about 300 niosomes were measured for the diameter. The average vesicular size of niosomes of all the batches was measured in the range of 4.40 μm to 5.77 μm . The result suggested that niosomes prepared were of uniform size and spherical in shape as shown in microphotographs Figure No.3 and Table No.3.

Surface morphology

Surface morphology of F1 formulation shown in figure results indicates that niosomal particles were appeared as discrete and round in shape with irregular surface due to the presence of unentrapped drug and observed mean particle size range as 10 μm (Figure No.4).

Zeta potential

The values of zetapotential of Flutamide loaded Niosomal formulation F1 were found to be -37.42 mV which are shown in Figure No.3. From the results we have observed that the formulations were sufficient to keep the particles stable.

In vitro drug release

In vitro drug release of various formulations of niosomes was shown in Figure No.5. The increase in surfactant (span 60) ratio from F1 to F4 causes increase in the drug release and the release was more

controlled by increasing the surfactant ratio. For F1-F4 the release were F1-74.50, F2-81.50, F3-84.84 and F5-87.00.

Release kinetics

To ascertain the drug release mechanism and release rate, data of the above formulations were model fitted using BCP dissolution software. The models selected were Zero order, Higuchi Matrix, Korsmayer Peppas. The regression coefficient values for all these models are shown in Table No.3. In all the cases the best fit model was found to be Higuchi with ‘n’ value below 0.5 suggesting the fickian release mechanism for the drug i.e., diffusion controlled. The results of model fitting were shown in Table No.4.

The study of drug release kinetics showed that majority of the formulations governed by Higuchi model. The curve was obtained after plotting the

cumulative amount of drug released from each formulation against time. Formulation F1 (74.50%) showed proper controlled release while other formulation showed more amount of drug release in 24hrs. Formulation F1 has correlation coefficient (r =0.9939) value and follows drug release by Higuchi model.

Stability studies

The intermediate stability study for F1 was performed for 6 months according to the ICH guide lines. Drug entrapment, vesicle size and drug release were fixed as physical parameters for stability testing and stability studies of selected formulation F1 showed that negligible changes in vesicle size, entrapment efficiency and drug release. This revealed that the formulation stable on storage at 4±2°C and 30±2 °C and 65±5% RH.

Table No.1: Composition of different niosomal formulation

S.No	Formulation code	Drug (mg)	Span-60 (mg)	Cholesterol (mg)	Phosphate buffer pH 7.4
1	F1	100	100	100	10 ml
2	F2	100	200	100	10 ml
3	F3	100	300	100	10 ml
4	F4	100	400	100	10 ml

Table No.2: Drug content and entrapment efficiency

S.No	Formulation code	% Entrapment efficiency	% Drug Content
1	F1	92.12	99.28
2	F2	90.32	98.34
3	F3	89.50	96.56
4	F4	89.96	97.89

Table No.3: Average vesicle size of F1-F8 formulation

S.No	Formulation code	Average vesicle size in µm
1	F1	4.40
2	F2	4.98
3	F3	5.13
4	F4	5.57

Table No.4: Datas for different kinetic model

S.No	Formulation code	Zero order	First order	Higuchi plot	Peppas plot	N values for Peppas
1	F1	0.7351	0.9881	0.9939	0.9851	0.4087
2	F2	0.8101	0.9842	0.9999	0.9856	0.4824
3	F3	0.8058	0.9852	0.9920	0.9925	0.4380
4	F4	0.8203	0.9824	0.9940	0.9606	0.4002

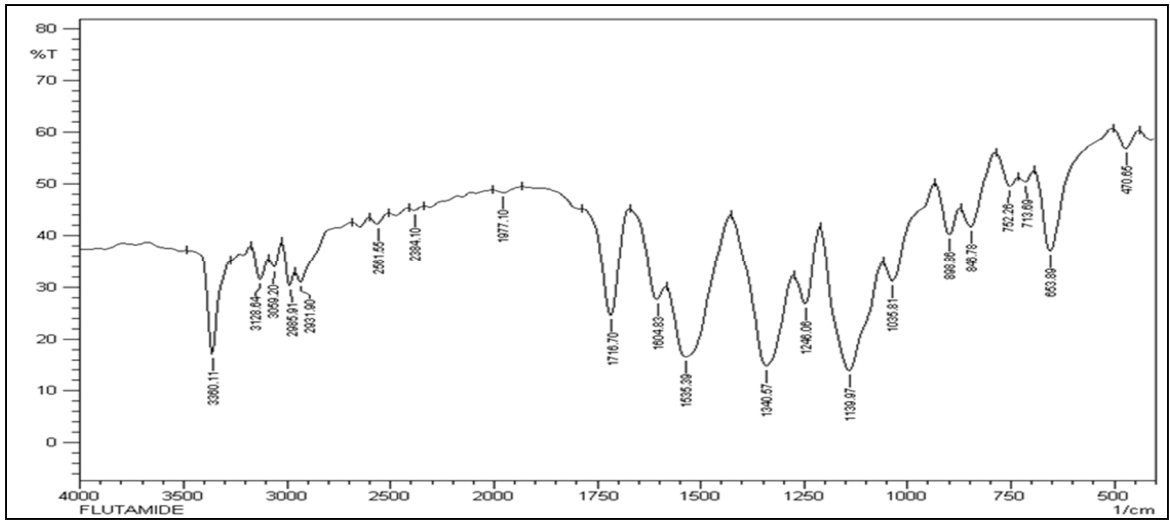


Figure No.1: FTIR Spectra of pure drug Flutamide

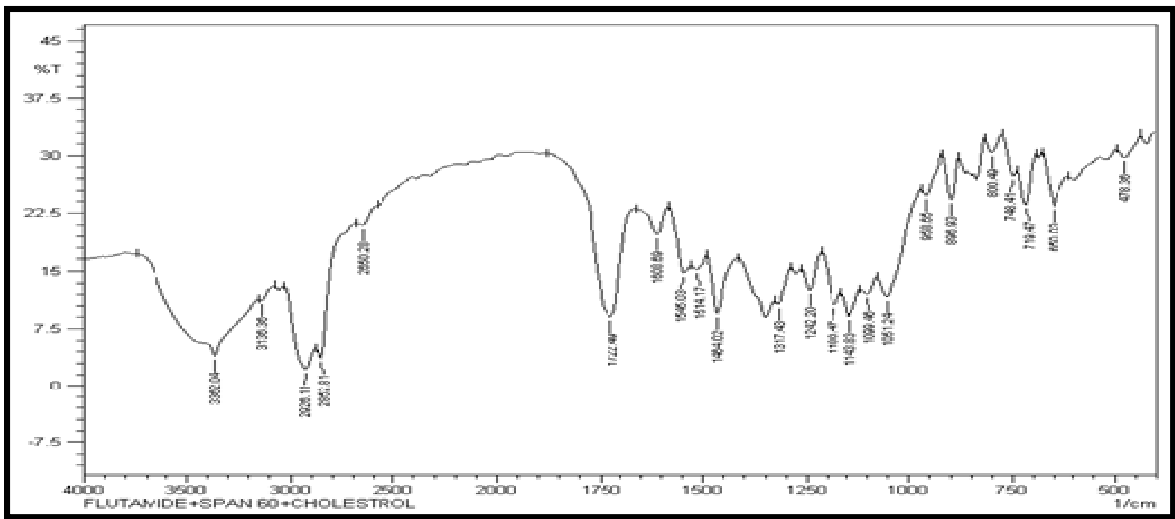


Figure No.2: FTIR Spectra of Flutamide+Cholesterol+Span 60

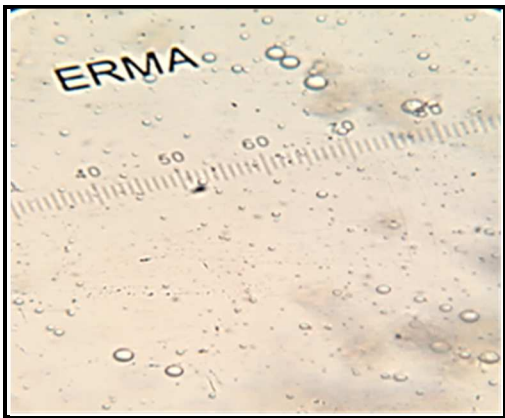


Figure No.3: Microphotographs of F1 formulation

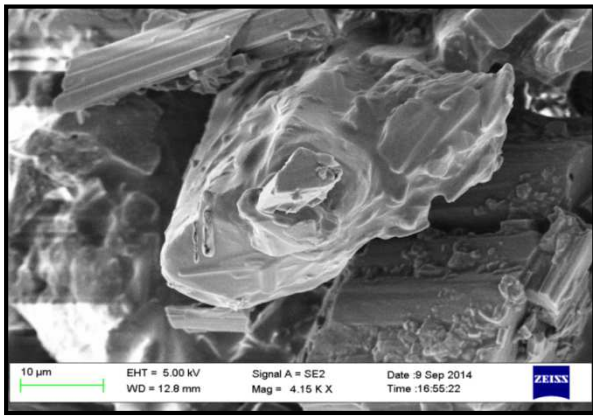


Figure No.4: SEM images of F1 formulation

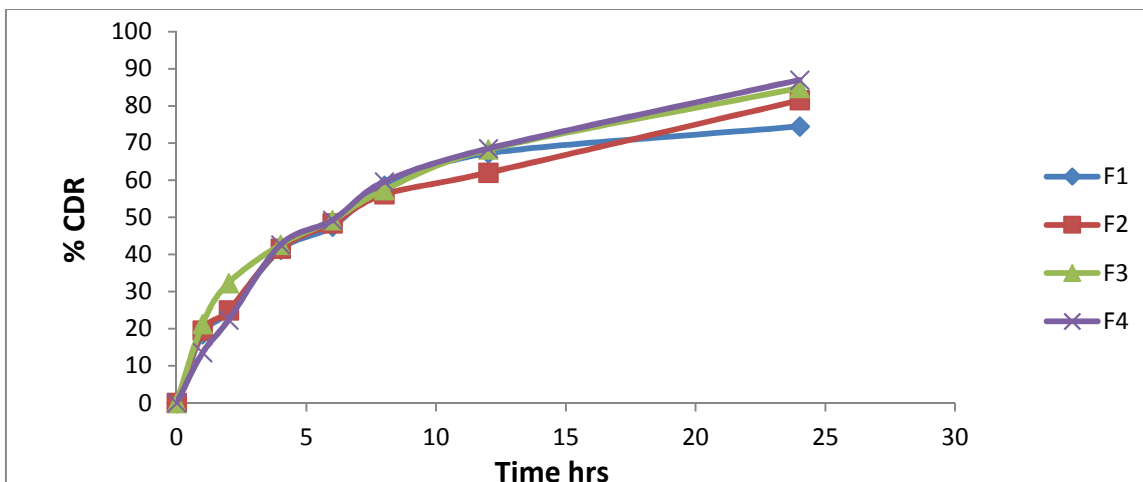


Figure No.5: In vitro release profile of F1-F4 formulation

CONCLUSION

The present study demonstrated the successful preparation of Flutamide niosomes and their evaluation. Formulation F1 showed high entrapment efficiency (92.12%), particle size (4.40 μm) and drug release (74.50%) over 24 hrs. Hence it was considered to be good niosomal formulation with greater bioavailability.

ACKNOWLEDGEMENT

The authors would like to thank Bharathi college of Pharmacy, Bharathinagara, Mandya, Karnataka, India for supporting for the fulfilment of this work.

CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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Please cite this article in press as: Navya M N. et al. The effect of span 60 in the formulation of niosomes containing flutamide, *International Journal of Research in Pharmaceutical and Nano Sciences*, 3(5), 2014, 444-449.