

Formulation and Evaluation of Atorvastatin Calcium Niosomes

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Received: 08 May 2016/Revised: 03 June 2016/Accepted: 21 June 2016

ABSTRACT- Atorvastatin calcium is a HMG-CoA reductase inhibitor used for the treatment of hyperlipidaemia. It has oral bioavailability of $\leq 12\%$. It also undergoes high first pass metabolism. It is highly soluble in acidic pH and absorbed more in the upper part of the gastrointestinal tract. In order, to improve the absorption and its oral bioavailability, niosomes of Atorvastatin calcium have been formulated and evaluated on different parameters. Four niosomes formulations of Atorvastatin calcium were successfully developed by modified ether injection technique using nonionic surfactant i.e. Span 20, Span 40, Tween 20, Tween 40 and cholesterol at different concentrations.

Key-words- Atorvastatin calcium, Niosomes, Surfactants, Cholesterol, Modified ether injection method, *in-vitro* release, Stability studies

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INTRODUCTION

The purpose of novel drug delivery system is to provide a therapeutic amount of drug to reach to the selected site in the body and then maintain the desired drug concentration to produce therapeutic effect. The drug- delivery system should deliver drug at a rate control by the necessarily of the body during a specific duration of treatment¹.

Niosome is a class of molecular cluster formed by self-association of non-ionic surfactants in an aqueous phase. The different structure of niosome presents an effective novel drug delivery system with ability of loading both hydrophilic and lipophilic drugs². Niosomes are promising vehicle for drug delivery and being non-ionic; and Niosomes are biodegradable, biocompatible non-immunogenic and exhibit flexibility in their structural characterization. Niosomes have been widely evaluated for controlled release and targeted delivery for the treatment of cancer, viral infections and other microbial diseases. Niosomes are one of drug delivery systems which have been employed as a substitute to liposomes. They are nonionic surfactant vesicles in aqueous medium resulting in closed bilayer structure that can be used as carrier of amphiphilic and lipophilic drugs³.

Major component of niosomes is non-ionic surfactant which give it an advantage of being more stable when compared to liposomes thus overcoming the problems associated with liposomes i.e. susceptibility to oxidation, high price and the difficulty in procuring high purity levels which influence size, shape and stability⁵. Niosomes serve as drug depot in the body which releases the drug in a controlled manner through its bilayer providing sustained release of the enclosed drug⁴.

Now a days Niosomes are gaining more popularity because of their stability, ease of preparation, achieving reduced toxicity, increasing drug efficacy and most importantly their site targeted action. These are important for drug targeting, particularly in chemotherapy, wherein, the limiting factor is the traditional lack of selectivity of drugs towards cancer cells and tissues. Niosomes are biodegradable, biocompatible and non-immunogenic. Niosomes can entrap both hydrophilic and lipophilic drugs and prolong the circulation of entrapped drug⁵.

Atorvastatin calcium is a HMG-CoA reductase inhibitor used in the treatment of hyperlipidaemia⁶. It has oral bioavailability of less than 12%. It also undergoes high first pass metabolism. It is highly soluble in acidic pH and absorbed more in the upper part of the GIT⁷. Atorvastatin is used for the lowering of serum cholesterol level. According to biopharmaceutical classification, it belongs to Class-II drug. It has very less solubility (<1 mg/ml), which limits its release from the formulation. It is primarily taken by the liver which results in low systemic bioavailability (14%) due to the first pass metabolism by the liver⁸.

In order, to improve the absorption and its oral bioavailability, niosomes of Atorvastatin calcium have been

Access this article online	
Quick Response Code:	Website: www.ijlssr.com
	DOI: 10.21276/ijlssr.2016.2.4.23
ISSN 2455-1716	

formulated and evaluated on different parameters.

MATERIALS AND METHODS

Atorvastatin calcium was received as gift sample from Shri Krishna Pharma Ltd, Hyderabad, Span 20, Span 40, Tween 20, Tween 40 (Qualikems Fine Chem Pvt Ltd., Varodara, India), Cholesterol (Loba Chem. Pvt ltd, Mumbai). All other chemicals were of analytical grade. Research work was carried out in Department of pharmaceuticals, Global Institute of Pharmaceutical education and Research, Kashipur, Uttarakhand for a total period of 6 months.

Formulation development by modified ether injection technique

Niosomes containing Atorvastatin calcium were prepared by modified ether injection technique using nonionic surfactant and cholesterol at different concentrations. Cholesterol and surfactant were dissolved in 6 ml diethyl ether mixed with 2ml methanol containing weighed quantity of Atorvastatin calcium. The resulting solution was slowly injected using micro syringe at a rate of 1 ml/min into 15 ml of hydrating solution phosphate buffer (pH 7.4). The solution was stirred continuously on magnetic stirrer and temperature was maintained at 60-65°C. As the lipid solution was injected slowly into aqueous phase, the differences in temperature between phases cause rapid vaporization of ether, resulting in spontaneous vesiculation and formation of niosomes⁹.

Different batches of niosomes were prepared in order to select an optimized formula as per general method described above and proportion of surfactant and cholesterol for the preparations of niosomes is given in Table-1.

Table 1: Compositions of the Atorvastatin calcium niosomes formulations

S.N.	Formulation code	Surfactant	Drug: surfactant: cholesterol (mg)
1.	NS1	Span 20	100:100:100
2.	NS2	Span 40	100:200:100
3.	NS3	Tween 20	100:100:100
4.	NS4	Tween 40	100:200:100

Evaluation of formulations

Scanning Electron Microscopy¹⁰

The shape and surface characteristics of niosomes were evaluated by Scanning Electron Microscopy (SEM). The lyophilized sample was mounted directly on to the sample holder using double-sided sticking tape and after gold coating images were recorded at the required magnification.

Particle size¹¹

Vesicle size determination was carried out using an optical microscopy with a calibrated eyepiece micrometer. About 200 niosomes were measured individually, average was taken, and their size range, mean diameter were calculated.

Entrapment efficiency¹²

The percentage entrapment efficiency of the vesicles was determined by freeze thawing centrifugation technique. Niosomal suspension was filled in drop tubes and stored at -20°C in a refrigerator for 24 hours. After 24 hours niosomal suspension was taken from refrigerator and stored at room temperature. The niosomal suspension was centrifuged at 1500 X G rpm for 30 minute. Supernatant containing untrapped drug was withdrawn and diluted with water methanol mixture (80:20), then measured UV spectrophotometrically at 244 nm against water methanol mixture as standard.

Entrapment efficiency (EE) was calculated by using following equation:

$$(\%)EE = \frac{\text{Total amount of drug in suspension} - \text{drug in supernant}}{\text{Total Amount of Drug present in suspension}} \times 100$$

In-vitro drug release¹³

The *in-vitro* permeation of Atorvastatin from niosomal formulation was studied using locally fabricated diffusion cell. The *in-vitro* diffusion of the drug through egg membrane was performed. It was clamped carefully to one end of the hollow glass tube of 17 mm (area 2.011 cm²) (dialysis cell) this acted as donor compartment. 100 ml of phosphate buffer saline PBS 7.4 was taken in a beaker which was used as a receptor compartment. The known quantity was spread uniformly on the membrane. The donor compartment was kept in contact with the receptor compartment and the temperature was maintained at 37± 0.1°C. The solutions of the receptor side were stirred by externally driven Teflon-coated magnetic bars.

At predetermined time intervals, sample was withdrawn and replaced by 4 ml of PBS. The drug concentrations in the aliquot were determined at 244 nm against appropriate blank. This experiment was done in triplicate and average value was reported. In- vitro permeation studies were conducted for different formulation and effect of variation in composition on permeation rate. Drug release data was normalized by converting the drug concentrations in solution to a percentage of cumulative drug release and was shown graphically.

Stability study¹⁴

Stability studies carried out by storing the prepared niosomes of Atorvastatin calcium at various temperature conditions like refrigeration on (2-8°C) room temperature (25±0.5°C) and elevated temperature (45±0.5°C) for a period of 12 weeks. Drug content and variation in the average vesicle diameter were periodically monitored. ICH

(International Conference on Harmonisation) guidelines suggests stability studies for dry niosomes powder meant for reconstitution should be studied for accelerated stability at 75% relative humidity as per international climatic zones and climatic conditions.

RESULTS AND DISCUSSION

A successful attempt was made to formulate twelve formulations of Atorvastatin calcium niosomes formulations by modified ether injection technique using nonionic surfactant and cholesterol at different concentrations. The pure drug shows sensitivity to light and moisture. Therefore formulating it into niosomes can solve this problem to a large extent.

The mean particle diameter of the Atorvastatin calcium niosomes was between 2.30-2.48 µm for all twelve formulations. Particles of all formulations were smooth, oval and discrete. Niosomes formulations of batch NS3 prepared by span 4o shows maximum mean particle diameter i.e. 2.50 µm. The entrapment efficiency of the niosomes was between 67.42-80.32%. The entrapment efficiency was found to be higher with the batch NS4 (80.32 %), which may have an optimum surfactant cholesterol ratio to provide a high entrapment of Atorvastatin calcium. The higher entrapment may be explained by high cholesterol content (~50% of the total lipid). It was also observed that very high cholesterol content had a lowering effect on drug entrapment to the vesicles. This could be due to the fact that cholesterol beyond a certain level starts disrupting the regular bi-layered structure leading to loss of drug entrapment.

Table 2: Properties of Atorvastatin calcium niosomes

S.N.	Formulation Code	Average mean diameter of non sonicated niosomes (µ m) ^a	% Entrapment efficacy ^a	Viscosity (Centipoise)
1.	NS1	2.48±0.12	67.42±0.21	2.036
2.	NS2	2.45±0.14	69.31±0.13	2.583
3.	NS3	2.50±0.09	70.42±0.14	2.347
4.	NS4	2.30±0.21	80.32±0.23	3.422

^a Average ± SD of three determination has been reported

Scanning Electron Microscopy morphology and internal cross-sectional structure of the microspheres were investigated with a Scanning Electron Microscope. SEM is one of the common methods used owing to the simplicity of sample preparation and ease of operation. Scanning Electron photomicrographs of the selected formulation shown in Fig. 1. SEM indicates that the niosomes were spherical with a smooth surface; distinct pores were evident on the surface of niosomes, which will be responsible for the release. The photomicrographs also showed presence of

loose crystals of drug on the surface of a few niosomes.

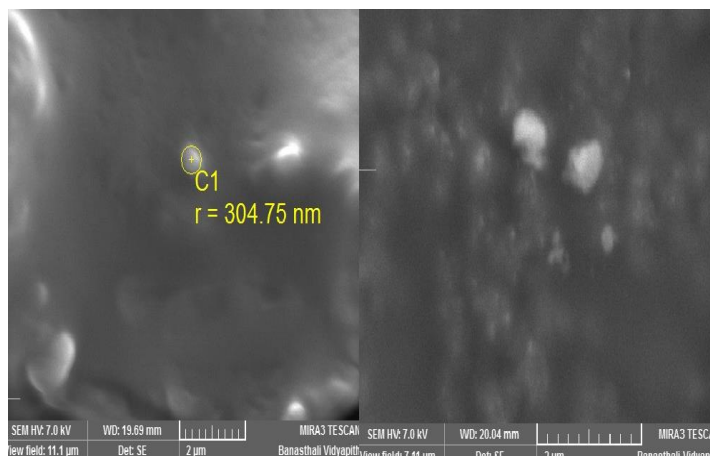


Fig. 1: SEM photograph of Atorvastatin calcium niosomes of batch NS1 and NS4

The *in-vitro* permeation of Atorvastatin calcium from niosomal formulation was studied using locally fabricated diffusion cell. The cumulative percent drug release after 11 hrs of the Atorvastatin calcium niosomes in between 32.46-84.64 %. The formulation containing tween showed less permeation compared with the preparation containing span. It might be due to the larger size of the vesicles and less lipophilic nature of the Tween, which makes it more difficult for these vesicles to penetrate or fuse with skin whereas, the inclusion of span which is more lipophilic than tween further increased the lipophilicity of the drug leading to better penetration.

Rapid drug leakage was observed during the initial phase. However, after that a slow release occurred. This could be because the drug is mainly incorporated between the fatty acid chains in the lipid bilayer of niosomal vesicles which leads to rapid ionization and release upon dispersing niosomes in large buffer (pH 7.4) volumes until reaching equilibrium.

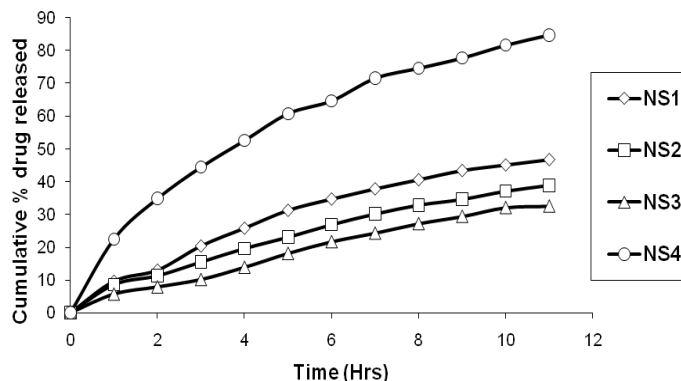


Fig. 2: Percentage of drug released from Atorvastatin calcium niosomes

Stability studies revealed that the niosomes kept at room temperature (~25°C) and 40°-75% RH showed the maximum stability. The values of drug content and *in-vitro*

studies were close to that of the initial data with only slight variations suggesting that it has an acceptable shelf life. It should be stored in a cool, dry place. Niosomes formulations of Atorvastatin calcium of batch NS4 shows good stability at refrigeration and room temperature in comparison to other.

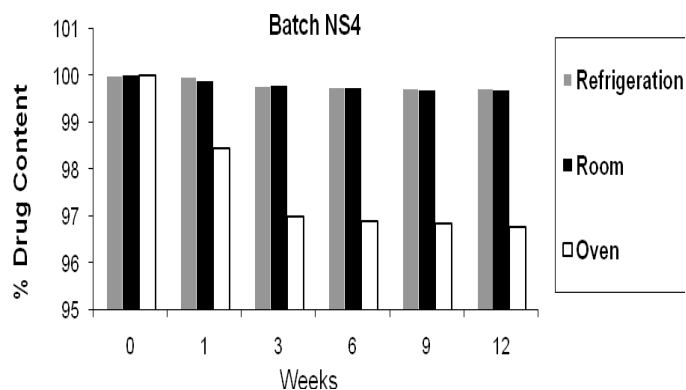


Fig. 3: Stability study of Atorvastatin calcium niosomes of batch NS4 at different temperature

CONCLUSIONS

At present scenario, transporting the drug molecules to the desired site in the biological systems has become a very specific and sophisticated area of pharmaceutical research. The role of the drug delivery system is not only limited to a drug package just meant for convenience and administration but to bring a required change in therapeutic efficacy and safety by carrying the drug molecules to the desired site in the most convenient manner. Atorvastatin calcium is a lipid lowering-agent and widely used to treat hypercholesterolemia and it is a potent inhibitor of HMG-CoA reductase. Four Atorvastatin calcium niosomes formulations were successfully developed by using different surfactants i.e. Span 20, Span 40, Tween 20, Tween 40 by modified ether injection method. Accelerated stability studies for 12 weeks revealed that the 12 Atorvastatin calcium niosomes formulations were stable at up to 45°C. The stability study of the optimized formulation showed satisfactory characteristics without being drastically influenced. On basis of drug content, particle size morphology, in-vitro release and stability studies, it can be concluded that formulation NS4 was an optimum formulation. However, future experiments should explore the suitability of niosomes with wide variety of drugs having designed drawbacks for improved and effective intended therapy. So, that niosomes are represented as promising drug carriers and promising drug delivery module.

ACKNOWLEDGMENT

The authors are thankful to Dr AK Saxena, Ex-Chief Scientist and former Head, Division of Medicinal Chemistry, CDRI, Lucknow, India for their technical suggestion and motivation during the work.

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Source of Financial Support: Nil

Conflict of interest: Nil