Research Article

FORMULATION, DEVELOPMENT AND CHARACTERIZATION OF ETHOSOMES OF ATORVASTATIN

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ABSTRACT

The present research purpose was to develop transdermal delivery of Atorvastatin, a HMG-CoA reductase inhibitor used in the treatment of congestive heart failure (CHF). Atorvastatin was formulated into ethosomal formulation to reduce the first pass metabolism and increase the systemic bioavailability. Differential light scattering and Transmission Electron Microscopy methods were utilized for the determination of entrapment efficiency of the ethosomal formulation. Vesicle morphology was studied and particle size was analysed for the characterization of all the ethosomal formulation. The in vitro release study of atorvastatin ethosomal formulation was determined by utilizing synthetic semipermeable membrane in franz diffusion cell. The optimized ethosomal formulation showed 22 times higher transdermal flux $34.5 \pm 0.4 \mu\text{g/cm}^2/\text{h}$ across synthetic semipermeable membrane as compared to plane atorvastatin drug formulation that is $3.6 \pm 0.5 \mu\text{g/cm}^2/\text{h}$. The result of in vitro release and characterization studies revealed that atorvastatin ethosomal formulation can deliver the drug more efficiently than conventional dosage form with no first pass metabolism and for a prolonged period of time.

Keywords: Centrifugation, Scattering, Transdermal, Ethosomes

INTRODUCTION

The technique of delivering a drug had a remarkable effect on its therapeutic efficacy. The therapeutic range of a drug is that at which it shows maximum therapeutic effect and dose above or below this concentration can be toxic or give no therapeutic effect at all. Due to retarded treatment of severe diseases, there is a growing demand of associative approach for the delivery of drug to targets in tissues. To decrease drug degradation, to enhance drug bioavailability and to arrest harmful side-effects there is a need to reinforce advanced drug targeting systems and various drug delivery for the faith of human life. Novel and Controlled Drug Delivery system are now an approach to provide a new life to the existing drug molecule. The various approaches for targeting the drug are niosomes, nanoparticles, ethosomes, liposomes, microparticles, microcapsules and lipoproteins etc.^{1,2} As compared to oral drug delivery system, transdermal drug delivery system present favourable result as it abolish first pass metabolism and gastrointestinal disturbances of the therapeutic moiety. For TDDS stratum corneum is the main barrier which is required to be encountered as it allow passage to only lipophilic drugs having molecular weight < 500 Da. Advanced technique involving use of permeation enhancers are now popularly utilized to enhance the drug permeability across the skin easily. Now a days as compared to liposomes which are widely used to deliver drugs to the outer layers of skin, ethosomes and transferosomes are popularly employed to appreciate permeation across the stratum corneum barrier.³

Ethosomes

“Ethosomes are ethanolic liposomes”. Ethosomes can be defined as noninvasive delivery carriers that enable drugs to reach deep into the skin layers and/or the systemic circulation. Ethosomes are

utilized mainly for the enhanced delivery of medicinal agent across the skin membrane. In return to the need of advanced drug delivery system Touitou invented a vesicular system called ethosomes for topical drug delivery across the skin. Ethosomes are soft, smooth, malleable vesicles utilized for novel delivery of medicaments.

Atorvastatin (Lipitor) is a member of the drug class known as statins, it is a HMG-CoA reductase inhibitor. It is used for lowering cholesterol. Atorvastatin is a competitive inhibitor of hydroxymethylglutaryl-coenzyme A (HMG-CoA) reductase, the rate-determining enzyme in cholesterol biosynthesis via the mevalonate pathway. Atorvastatin acts primarily in the liver. The absolute bioavailability of atorvastatin is approximately 14% and the systemic bioavailability of HMG-CoA reductase inhibitory activity is approximately 30%. The low systemic bioavailability is due to presystemic clearance by gastrointestinal mucosa and first pass metabolism in the liver. Atorvastatin is most widely used in coronary heart disease (CHD).^{4,5}

MATERIAL AND METHOD

Atorvastatin was obtained as a gift sample from Chandra Labs, Hyderabad, India. Soya lecithin and other ingredients were obtained as a gift sample from Fine Chem Industries, Mumbai. Cholesterol was purchased from Cortex Laboratories, Hyderabad. All the other raw materials were of pharmacopoeial grade.

Pre-formulation study: Standardization of the drug was carried out using phosphate buffer pH 6.8 by UV spectrophotometer (UV-160A, SHIMADZU). Solubility analysis of drug in various solvents including water, phosphate buffer pH 6.8, and organic solvents like methanol was carried out.

Formulation of Ethosomes

Screening of formulation component with Atorvastatin

The foremost step was to screen different formulation component with Atorvastatin. In this step we screened different components with the drug Atorvastatin for the final selection of the excipients for the development of ethosomes of drug by cold method. The preliminary trial batches of ethosomes were prepared using selected process parameters to check the feasibility of preparation of ethosomes by using the available excipients that were also reported in earlier studies.⁶

Drug-polymer interaction

FTIR (Fourier Transform Infrared Spectroscopy): To determine the drug-polymer interaction IR spectra of physical mixture of drug and polymers in combination were taken.

Formulation of Ethosomes as drug carriers by cold method

Atorvastatin loaded ethosomes were prepared by cold method followed by ultra sonication. Phospholipids, drug and other lipid material were dissolved in ethanol in a covered beaker at a room temperature by vigorous stirring with the use of magnetic stirrer. Propylene glycol was added during stirring. This mixture was heated to 30°C in a water bath. The water heated to 30°C in a separate beaker was added to the mixture which was then stirred for 5 min in a covered vessel using magnetic stirrer. The vesicle size of ethosomal formulation was then reduced by ultrasonic probe sonicator for 10 min and off time was 10 sec. Finally the formulation was stored under refrigeration. The vesicles were observed under Carl Zeiss trinocular microscope at 40 X and 100 X magnification.⁷

Characterization of optimized ethosomal drug delivery systems

pH: The pH of the prepared formulations after addition of all the ingredients was measured by using digital pH meter.

Drug – Carrier Interaction Study

Fourier Transformed Infrared Spectroscopy: Fourier Transformed Infrared Spectroscopy of samples and Pure drug i.e Atorvastatin were attained using FTIR spectrophotometer.

Vesicle shape: Ethosomes can be easily visualized by using transmission electron microscopy (TEM) or by differential scanning calorimetry DSC.

Elasticity measurement

Extrusion method was utilized to measure the elasticity of ethosomal formulation. A filter membrane was utilized to analyse the elasticity of vesicles in which the ethosomal vesicles were allowed to pass through it under the 3.4 bar pressure and in each 5 minute interval the extruded vesicle suspension amount was determined.

Vesicle size and zeta potential: In this method we determined the size of ethosomal preparation by using Dynamic Light Scattering technique with the help of Malvern Autosizer 5002 inspection system. Zeta potential of the formulation was measured by Malvern zeta sizer.

Drug entrapment: The entrapment efficiency of ethosomes can be measured by the ultracentrifugation technique. The entrapment

efficiency (EE), of Atorvastatin ethosomes, was determined by measuring the concentration of free Atorvastatin in the dispersion medium. 1.0 ml of the ethosomes was diluted up to 10 ml with phosphate buffer (pH 5.5) and centrifuged at 14000 rpm for 1 hour to separate the lipid and aqueous phase. Supernatant was then filtered by 0.2µ membrane filter and analyzed by UV-VIS spectroscopy at 239 nm.

$$\% EE = [\text{Initial drug} - \text{Free drug}] \times 100 / \text{Initial drug}$$

Where,

Initial drug = mass of initial drug used for the assay.

Free drug = mass of free drug detected in the supernatant after centrifugation of the aqueous dispersion.

Drug content

Drug content of the ethosomes can be determined using UV spectrophotometer. This can also be quantified by a modified high performance liquid chromatographic method. Ethosomal suspension (1 ml) was taken and dissolved in 100ml methanol and shaken vigorously for 2 h in magnetic stirrer, there after the resultant solution was filtered and 1ml of sample was withdrawn and diluted to 10 ml in a volumetric flask and the absorbance was recorded in UV spectrophotometer at 239 nm.

In-vitro release studies

In-vitro drug release from the formulation was studied by the diffusion cell. Phosphate buffer pH (5.5) is used as diffusion medium, stirred at 50 rpm at 37°C ± 0.5°C. The 5 ml of ethosomal formulations were filled in test tubes and the end of the test tubes was covered with a dialysis membrane and was placed in such way that it touches the surface of diffusion medium present in receptor compartment. The drug sample were withdrawn at the interval of half an hour for the time period of 12 h from diffusion medium and analyzed by using UV spectrophotometer at 239 nm, using phosphate buffer pH (5.5) as a blank.⁸

RESULT AND DISCUSSION

Drug – Carrier Interaction Study

FTIR Study: It was found that there is no considerable change in the peak of the drug polymer mixtures when compared to pure Atorvastatin. FTIR spectra of the prepared drug polymer mixture shows that no change occurred in chemical structure. Thus it can be concluded that, according to FTIR studies there is no physico-chemical interaction between Atorvastatin and the polymers.

Drug entrapment: For entrapment efficiency total percentage of drug entrapped was determined in both cases in traditional dosage form as well as in vesicular formulation. The data indicate that entrapment efficiency depends on ethanol concentration, as the concentration increases up to 30%, it results in increase in entrapment efficiency of ethosomal formulation. With further increase in ethanol concentration entrapment efficiency decreases, owing to increase in fluidity of membrane and vesicles become more permeable that leads to decrease in entrapment efficiency of ethosomal formulation.

In-vitro release studies

From the in-vitro drug release studies we concluded that ethosomal vesicles present a efficient system for delivering the drug across the skin along with the use of penetration enhancer which also enhances the delivery across the skin with increased bioavailability.

TABLE 1: LIST OF EXCIPIENTS USED

S.No	Excipients
1	Phosphatidylcholine
2	Phosphatidylethanolamine
3	Cholesterol
4	Polyethylene glycol
5	Phosphatidylinositol

TABLE 2: FORMULATION OF ATORVASTATIN ETHOSOMES

S.No.	Ingredient	Formulation code							
		E1	E2	E3	E4	E5	E6	E7	E8
1	Atorvastatin	20	20	20	20	20	20	20	20
2	Soya Lecithin	15	15	15	30	30	30	45	60
3	Cholesterol	15	30	45	15	30	45	15	45
4	Ethanol	6	6	6	9	9	12	12	15
5	Propylene glycol	3	3		3	3	3	3	3
6	Distilled water	Upto 30ml	Upto 30ml	Upto 30ml	Upto 30ml	Upto 30ml	Upto 30ml	Upto 30ml	Upto 30ml

TABLE 3: ELASTICITY OF ATORVASTATIN ETHOSOMAL FORMULATIONS (SD= STANDARD DEVIATION)

Formulation Code	Elasticity $\pm$ SD
E1	84.72 $\pm$ 5.46
E2	80.93 $\pm$ 7.32
E3	86.07 $\pm$ 5.2
E4	86.4 $\pm$ 4.67
E5	89.9 $\pm$ 5.77
E6	84.68 $\pm$ 7.44
E7	84.33 $\pm$ 3.22
E8	83.91 $\pm$ 3.21

TABLE 4: PARTICLE SIZES OF ATORVASTATIN ETHOSOMAL FORMULATIONS. (SD= STANDARD DEVIATION)

Formulation Code	Particle size $\pm$ SD
E1	94.64 $\pm$ 0.45
E2	112.52 $\pm$ 0.46
E3	153.63 $\pm$ 0.41
E4	149.75 $\pm$ 0.19
E5	95.6 $\pm$ 0.78
E6	117.85 $\pm$ 0.52
E7	94.62 $\pm$ 0.76
E8	104.69 $\pm$ 0.98

TABLE 5: ENTRAPMENT EFFICIENCY OF ATORVASTATIN ETHOSOMAL FORMULATIONS. (SD= STANDARD DEVIATION)

Formulation	EE (%) $\pm$ SD
E1	97.8 $\pm$ 2.43
E2	96.44 $\pm$ 3.14
E3	98.58 $\pm$ 0.98
E4	97.48 $\pm$ 1.47
E5	98.65 $\pm$ 0.63
E6	96.45 $\pm$ 3.11
E7	98.04 $\pm$ 2.34
E8	96.65 $\pm$ 2.56

Fig 1: FTIR of Atorvastatin

Fig 2: FTIR of Atorvastatin + Carbopol

Fig 3: FTIR of Atorvastatin + Cholesterol

Fig 4: FTIR of Atorvastatin + Soya Lecithin

Fig 5: FTIR of Atorvastatin + Propylene glycol

Fig 6: Scanning electron microscopy of Atorvastatin Ethosomal preparation

Fig 7: Zeta potential of prepared Atorvastatin ethosome

Fig 8: %CDR of Atorvastatin ethosomal formulation E1, E2, E3 and E4

Fig 9: %CDR of Atorvastatin ethosomal formulation E5, E6, E7 and E8

Discussion

The studies showed that there is no interaction between drug and excipients, it means drug and excipients are compatible with each other. The studies also revealed that ethosomal formulation of atorvastatin shows better entrapment efficiency as well as drug release profile as compared to conventional dosage form for a prolonged period of time by eliminating the first pass metabolism

CONCLUSION

Based on the observation it was concluded that ethosomal formulation of atorvastatin is more effective than any other conventional formulation. The various formulation was analysed for different parameters like vesicle size, vesicle shape and entrapment efficiency and the result showed the potential of ethosomes of being a safe and very efficient drug carrier topical delivery of drug. The study also revealed that significant amount of atorvastatin was transported across the skin when entrapped in ethosomes. Thus ethosomal formulation were found to be promising candidate for transdermal delivery of atorvastatin with enhanced bioavailability.

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