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Research Article

FORMULATION AND CHARACTERIZATION OF ATORVASTATIN PHARMACOSOMES AS AN ALTERNATIVE APPROACH TO CONVENTIONAL VESICULAR SYSTEM

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ABSTRACT

The vesicular systems are the concentric lipid bi-layer assemblies that are formed when certain amphiphilic building blocks are confronted with water. This system extends the therapeutic index by encapsulating the drug molecules inside the vesicular structure. It prolongs the presence of drug molecules in systemic circulation and reduces the toxicity which in turn results in the modification of pharmacokinetics and bio distribution of drugs. Pharmacosomes serve as an alternative to conventional vesicles, have unique advantages over liposome, noisome, transferosomes etc. and are defined as colloidal dispersions of drugs covalently bound to lipids and may exist as ultrafine vesicular, micellar or hexagonal aggregates. The objective of the present study was to prepare and evaluate atorvastatin calcium pharmacosomes using two different ratios (1:1 and 1:2) of drug and phosphatidylcholine in the presence of dichloromethane by solvent evaporation technique. The results of physicochemical study showed higher solubility of almost tenfold increase in solubility of pharmacosomes formulation as compared to pure drug. The dissolution rate of phospholipid complex is also improved to 98% from 93%. Hence phospholipid complex of drug can be used as a potential tool of improving dissolution rate and bioavailability. The release data was fit into different kinetic models and it has been found to follow Higuchi model with a Fickian release mechanism in all the formulations.

Keywords: Pharmacosomes, Atorvastatin, Bioavailability, Vesicular systems

INTRODUCTION

Pharmacosomes are colloidal dispersion of drug covalently bound to lipid. They may exist as an ultra-fine vesicular, micelle or hexagonal aggregates depending upon the chemical structure of drug-lipid complex. The name is given recently to certain vesicular drug carrier systems where the drug is conjugated with the vesicle-forming agent, usually, palmitic / stearic acids or their derivatives. Thus, the problem of drug leakage and low entrapment efficiency, often associated with other vesicular system, is eliminated. Any drug with a certain cut-off molecular weight may be formulated as pharmacosomes provided it has active functional groups to integrate with the vesicle-forming amphiphilic molecule[1]. The primary use of Atorvastatin is for the treatment of dyslipidemia and the prevention of cardiovascular disease[2]. Pharmacosomes are suitable for both hydrophilic and lipophilic drugs, not influence entrapment efficacy. It can be given orally, topical, intravascular with reduction of cost of therapy and adverse effects and toxicity[3].

Pharmacosomes shows better biopharmaceutical properties to the drug result in improved bioavailability. The phospholipid complex of Atorvastatin calcium may also be useful for minimizing the GI toxicity of Atorvastatin calcium[3]. Although pharmacosome potentially improve the solubility and dissolution rate, it may also be used in drug targeting and in control release of the drug. The development of pharmacosome is dependent on surface and bulk interactions of lipids with drugs. Drugs containing an active hydrogen atom (COOH,-OH,-NH, etc.) can easily be formulated as pharmacosome complex by esterifying with the lipid resulting in the formation of a compound suitable for penetrating into the target site of the therapy[4].

MATERIALS AND METHODS

Atorvastatin Calcium was obtained as a gift sample from Preet Remedies Pvt. Ltd, Baddi, Himachal Pradesh. Lecithin Soya was purchased from Hi Media Laboratories Pvt Ltd., India. Dichloromethane was purchased from Merck Private Limited, India. Potassium di hydrogen phosphate was purchased by Qualigens Fine Chemicals, India. Sodium hydroxide pellets purified was purchased from Central Drug House (P) Ltd., India.

Fourier Transform Infra-Red Spectroscopy (FT-IR) Study

To study the possible interactions between Atorvastatin Calcium and lecithin soya (30%), FT-IR spectroscopy was carried out for the pure atorvastatin calcium, lecithin soya (30%) as well as the physical mixture of atorvastatin calcium and lecithin soya (30%). The IR spectra were recorded using FT- IR Spectrophotometer (Perkin Elmer FT- IR, Perkin Elmer Inst. USA) by KBr pellet method.

Preparation of atorvastatin calcium pharmacosomes

The atorvastatin calcium pharmacosome was prepared by associating atorvastatin calcium with phosphatidylcholine in two different ratios (1:1 and 1:2). The accurately weighed atorvastatin calcium and lecithin soya 30% (phosphatidylcholine) were placed in a 100 ml round bottom flask and dissolved in dichloromethane. The mixture was then refluxed for 1 hour and then lyophilized. After completion of drying; the dried residue was collected and placed in a vacuum desiccator for overnight and then subjected to characterization. The prepared atorvastatin calcium pharmacosomes were shown in Table 1.

Table 1: Formulation of Atorvastatin calcium Pharmacosome

Formulation	Formulation code	Atorvastatin Calcium : Lecithin soya (30%)
Free atorvastatin calcium	PURE ATOR	1:0
Atorvastatin Calcium Pharmacosome (1:1)	ATOR PH (1:1)	1:1
Atorvastatin Calcium Pharmacosome (1:2)	ATOR PH (1:2)	1:2

Solubility study

To determine the change in solubility due to complexation, the apparent solubility of atorvastatin calcium and atorvastatin calcium pharmacosomes were determined by adding an excess amount of drug and pharmacosomes to 5 ml distilled water in respective centrifuge tubes. The tubes were then allowed to shake and dissolved the maximum amount of drug and pharmacosomes in respective centrifuge tubes and then tubes were subjected to sonication. After attaining equilibrium, the saturated solutions were centrifuged to remove excess drug. The supernatant was filtered, and suitable dilutions were made with same solvent and then analyzed spectrophotometrically at 247 nm.

***In vitro* dissolution study**

In vitro dissolution studies for atorvastatin calcium pharmacosomes as well as pure atorvastatin calcium were performed in USP dissolution test apparatus at 50 rpm and at 37^0C. An accurately weighed amount of pharmacosomes equivalent to 20 mg of drug and 20 mg pure drug were placed in 900 ml media (distilled water). Samples (5 ml each) of dissolution fluid were withdrawn at different time intervals and replaced with the equal volume of fresh media to maintain sink conditions. Withdrawn samples were filtered and diluted suitably and then analyzed spectrophotometrically (Shimadzu UV-1700, Japan) at 247 nm.

Drug content study

To determine the drug content in pharmacosomes of atorvastatin calcium, a complex equivalent to 10 mg atorvastatin calcium was weighed and added into a volumetric flask with 100 ml of distilled water. Then the volumetric flask was stirred continuously for 24 hours on a magnetic stirrer. At the end of 24-hour, suitable dilutions were made, and drug contents were measured at 247 nm spectrophotometrically[5] (Shimadzu UV-1700, Japan).

Scanning electron microscopy (SEM)

To detect the surface morphology of the pharmacosomes, SEM of the complex was recorded on a scanning electron microscope (Model – JEM 6360, JEOL make, UK). The samples and an appropriate amount of pure drug were fixed on an SEM brush stub using double-sided carbon tape and coated with palladium (coating thickness 30 nm). A scanning electron microscope with a secondary electron detector was used to obtain digital images of the samples at an accelerating voltage of 17 kV.

Differential scanning colorimetry (DSC)

Differential scanning calorimetric analysis was performed using Shimadzu DSC-60 system. Sample of Atorvastatin Calcium pure drug, Lecithin soya (30%) and the Atorvastatin Calcium pharmacosomes were placed in a sealed aluminum pan. The prepared samples (4-7 mg) were heated at a rate of 10^0 C/min in 20^0 C -350^0 C range, using an empty sealed pan as a reference. Enthalpy changes (ΔH) were calculated from peak areas of samples and to study the polymorphic changes in the formulations.

X-ray diffraction (XRD) study

The crystalline state of atorvastatin calcium in the different samples was evaluated using X-ray powder diffraction. Diffraction pattern were obtained on X-ray diffractometer (Model – Ultima III; Rigaku make, Japan). The X-ray generator was operated at 40 kV tube voltages and 30 mA tube current, using the K a line of copper as the radiation source. The scanning angle ranged from 1 to 80^0 of 2Θ in the step scan mode (step width 5^0 min-1). Atorvastatin Calcium, lecithin soya (30%), and pharmacosomes were analyzed with X-ray diffractions.

Stability Studies

The optimized pharmacosomal formulation was selected and stored in tightly closed glass vials at room temperature and in refrigerator (4 ± 2ºC). Then each sample was withdrawn at an interval of one week and analyzed to determine the leakage rate. The samples were analyzed at predetermined time intervals visually and under optical microscope for appearance of drug agglomerates.

RESULTS AND DISCUSSION

Fourier transforms infra-red spectroscopy (FTIR) study

The FT-IR spectrum of pure Atorvastatin calcium showed all the characteristic bands of drug, including the carbonyl stretching at 3363.86 cm^{-1} , which corresponds to COOH functional group of Atorvastatin calcium. Lecithin soya (30%) has CO, O, and reactive NH_2 group and it showed the peaks at 3414.83 cm^{-1}, 2972.55 cm^{-1}, 2359.81 cm^{-1}, 1787.33 cm^{-1} , 1563.09 cm^{-1}. Peaks observed in pure drug have remained unaffected in the physical mixture. Thus, it can be concluded that the drug-soya lecithin physical mixture does not show any major interactions[6].

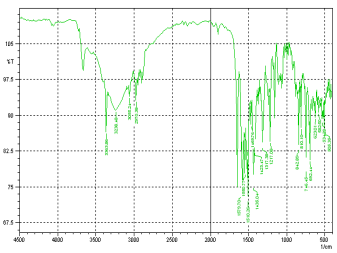


Figure 1A

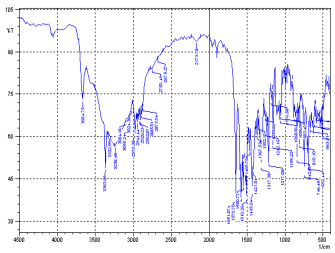


Figure 1B

Figure 1: FT-IR spectrum of (A) Atorvastatin Calcium, (B) Physical mixture of Atorvastatin Calcium and Lecithin Soya (30%)

Drug content study

Drug content of Atorvastatin calcium in the pharmacosomes was determined by UV spectrophotometer at 247 nm in distilled water and the drug loading was found 95.89 % (w/w) in pharmacosomes (1:1) and 89.78 % in pharmacosomes (1:2). The pharmacosomes showed a high percent of drug loading.

Solubility study

Solubility of Atorvastatin in pharmacosomes was found to be much higher than that of free Atorvastatin calcium. The Atorvastatin calcium pharmacosomes showed amphiphilic nature, which may prove to be responsible for improved bioavailability of the drug[7].

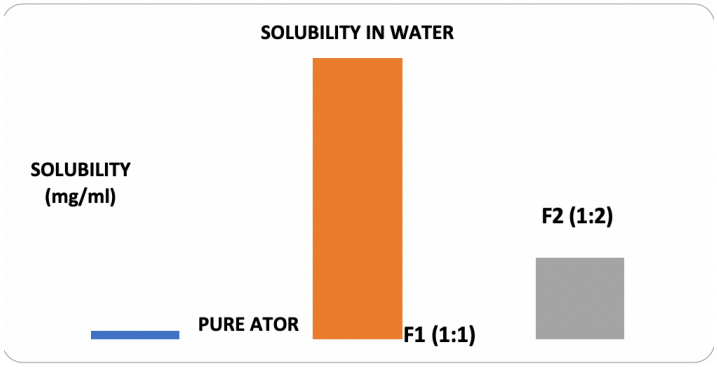


Figure 2: Solubility of Atorvastatin calcium in distilled water

***In vitro* dissolution study**

The free Atorvastatin calcium showed only 83.14% drug release at the end of 1.25-hour dissolution study while Atorvastatin calcium pharmacosome (1:1) showed 98.11% and Atorvastatin calcium pharmacosome (1:2) showed 95.49 % at the end of 1.25-hour dissolution study in distilled water.

Differences in the crystal habit, surface area, surface energies, particle size and wettability may all play a role in affecting the dissolution rate of powder[8].

The release data was fitted into various models, namely, Zero order, First order, Higuchi model and Korsmeyer-Peppas model. The regression coefficient values were calculated for each model and the Higuchi equation was found to be better fit as compared to other model.

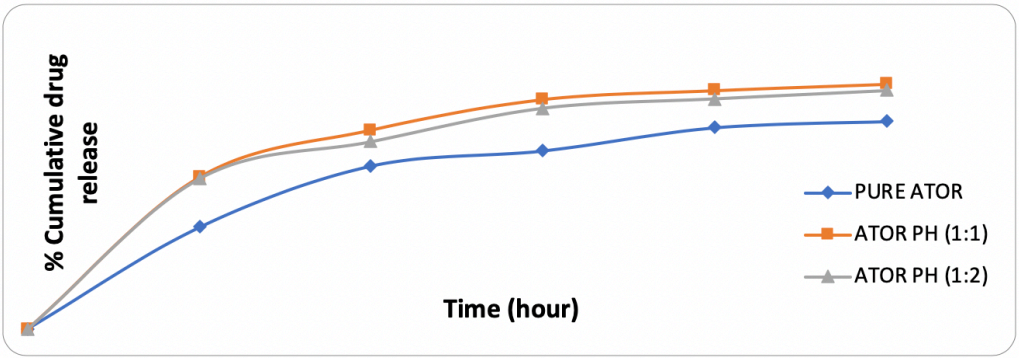


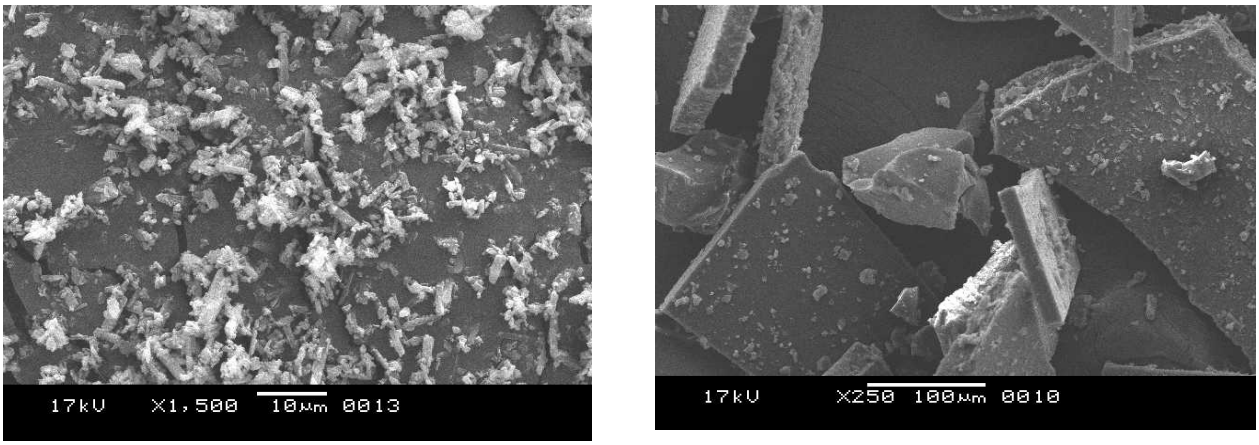
Figure 3: *In Vitro* Drug Dissolution Profile (Zero order) of Pure Atorvastatin Calcium and Atorvastatin Calcium Pharmacosomes

Table 2: Comparison of release kinetics of different Atorvastatin calcium pharmacosomes formulation and pure Atorvastatin Calcium

Formulation code	Zero order (r^2)	First order (r^2)	Higuchi model (r^2)	Korsmeyer-Pepas (r^2)	n value	Best fit	Release pattern
Pure ATOR	0.707	15.7	0.976	183	80.52	Higuchi model	Fickian
ATOR PH (1 :1)	0.541	2.12	0.937	419	99.14	Higuchi model	Fickian
ATOR PH (1 :2)	0.549	4.32	0.941	447	95.62	Higuchi model	Fickian

Scanning electron microscopy

From the Figure 4(A) Atorvastatin calcium appeared to be made of several smooth rectangular crystalline structures and pharmacosomes 4(B) were found to be irregular or disc shaped or rough surface morphology. Several Crystalline structures characteristics of solid Atorvastatin calcium are not seen in pharmacosome micrographs suggesting that the drug is present in an amorphous or molecularly dispersed state in the pharmacosomes[5].



(A) (B)

Figure 4: Scanning electron micro graphy of Atorvastatin Calcium (A) and Pharmacosomes (B)

Differential scanning colorimetry

The formation of a complex of Atorvastatin calcium and lecithin soya due to presence of unique peak in the complex (pharmacosomes) which were entirely different from the positions of the peaks of Atorvastatin calcium and lecithin soya. Atorvastatin calcium elicits a sharp endothermic peak at 131.50^{0}C while lecithin soya showed three major peaks at 157.98^{0}C, 1462.44^{0}C and 169.75^{0}C. It is evident that original peaks of Atorvastatin calcium and lecithin soya disappear from the thermo grams of pharmacosomes[9].

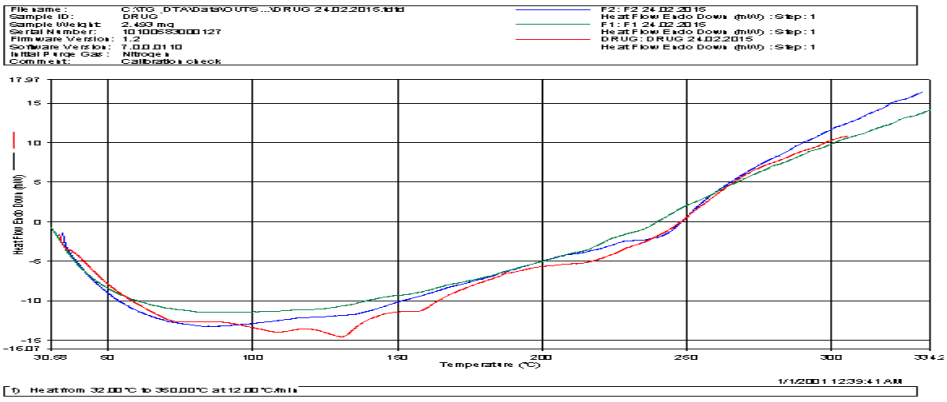
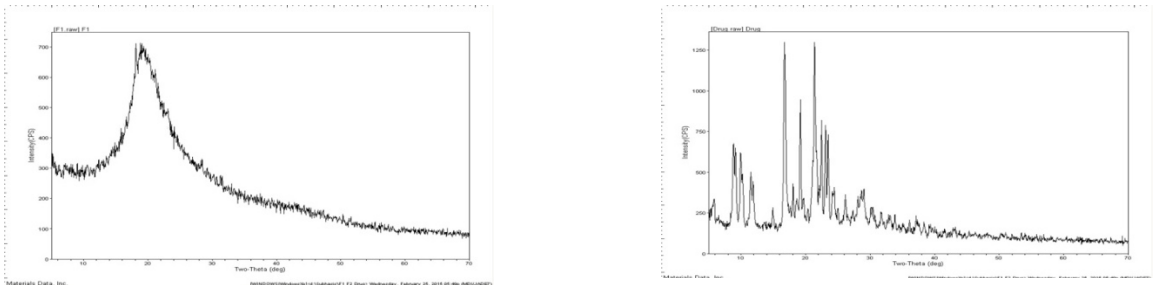


Figure 5: DSC Thermograms of (a) Atorvastatin Calcium (Red), (b) Atorvastatin Calcium Pharmacosome (1; 1) (Green), (c) Atorvastatin Calcium Pharmacosome (1; 2) (Blue)

X-ray diffraction (XRD) study

To determine the changes in crystalinity of the drug in the pharmacosomes X-ray diffraction analysis was conducted. The XRD patterns were shown in Figure 6.



(A) (B)

Figure 6: X-ray diffraction study of (A) Atorvastatin Calcium (B) Atorvastatin Calcium pharmacosome (1:1)

Calcium pharmacosomes revealed a broad peak similar to lecithin soya (30%). The formation of phospholipid complexes (pharmacosomes) were confirmed by the absence of crystalline diffraction peaks of Atorvastatin calcium[10]. The degree of crystalinity can be evaluated by patterns of changes in peaks.

CONCLUSION

In pharmacosome, drug (Atorvastatin) itself conjugates with lipids and forming vesicular delivery system. Atorvastatin pharmacosomes have not only high entrapment efficiency it also improves the solubility and dissolution rate of drug. Over and above it has immense potential and advantages over other vesicles. Hence it could be a better alternative over other vesicular drug delivery system.

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