Research Article

Formulation, Optimization and Evaluation of Multiple Emulsion of Atorvastatin

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ABSTRACT

Multiple emulsions are often stabilized using a combination of hydrophilic and hydrophobic surfactants. The ratio of these surfactants is important in achieving stable multiple emulsions. Atorvastatin was selected as a model drug to study the potential of multiple emulsions to improve bioavailability with the hypothesis that improvement of drug release profile will reflect the enhancement of bioavailability of the drug. The objective of this study was to prepare multiple emulsion of Atorvastatin by two step emulsification using non-ionic surfactants, and evaluate for stability, percentage drug entrapment, *In-Vitro* & *Ex-Vivo* drug release. The different variables like, rpm, concentration of surfactants and ratio of aqueous and oil phase were optimized to get the stable emulsion with high drug release and less particle size. The study concluded that stable multiple emulsion with high drug release can be prepared by two step emulsification method using Span80 as primary emulsifier at 30:70 phase volume ratio of internal phase: external phase with optimized speed of stirring at 3000 r/min for 15 mins for primary emulsification. The optimized formula were used for the preparation of multiple emulsion with the optimum drug release characteristics.

Keywords: Multiple Emulsion; Non-ionic Surfactant; Atorvastatin, Optimization.

INTRODUCTION

An ideal dosage regimen in the drug therapy of any disease is the one which immediately attains the desired therapeutic concentration of drug in plasma (or in the site of action) and maintains it constant for the entire duration of treatment. For many decades, treatment of an acute disease or a chronic illness has been mostly accomplished by delivery of drugs to patients using various pharmaceutical dosages forms, including tablets, capsules, pills, suppositories, creams, ointment ,liquids, aerosols, and injectable, as drug carriers. Even today these conventional drug delivery systems are the pharmaceutical products commonly seen in the prescription and over-the-counter drug market place. This type of drug delivery system is known to provide a prompt release of drug. Therefore, to achieve as well as to maintain the drug concentration within the therapeutically effective range needed for treatment. When administered into the body, this gives high therapeutic efficacy with minimal toxicity. It gives better selectivity of pharmacological activity, and improves patient compliance by reducing the dosing intervals.

Multiple emulsions are defined as emulsions in which both types of emulsions, i.e. water-in oil (w/o) and oil-in-water (o/w) exist simultaneously. They combine the properties of both w/o and o/w emulsions. These have been described as heterogeneous systems of one immiscible liquid dispersed in another in the form of droplets, which usually have diameters greater than 1 µm.

Multiple emulsions were determined to be promising in many fields, particularly in pharmaceutics and in separation science. Their potential biopharmaceutical applications include their use as adjuvant vaccines, as prolonged drug delivery systems, as sorbent reservoirs in drug overdose treatments and in mobilization of enzymes. Multiple emulsions were also investigated for cosmetics for their potential advantages of prolonged release of active agent, incorporation of incompatible materials and protection of active ingredients by dispersion in internal phase.[1]

Atorvastatin, a synthetic HMG Co-A Reductase inhibitor, is widely used in treatment of primary hypercholestremia and Dyslipidaemia. Atorvastatin is indicated as adjunctive therapy to diet for the treatment of patients with elevated serum triglyceride levels (Fredrickson Type IV). Oral bioavailability of Atorvastatin is very low (Only 14%) due to its presystemic clearance in gastrointestinal mucosa and first pass hepatic metabolism. However, very few studies have been reported for enhancement of bioavailability of poorly water soluble drugs by formulating as multiple emulsions.

The present study is based on the hypothesis that improvement of in vitro as well as ex vivo (using rat intestines) dissolution profile of AT and it will reflect the enhancement of bioavailability of the drug.

MATERIALS AND METHODS

Materials

Atorvastatin calcium was obtained as the gift sample from Sance laboratories pvt. limited, Kerala, Tween 80 from Merklimite, Mumbai, Span 80 from Chemdyes Corporation and light liquid paraffin from Spectrum reagents chemicals. All the reagents and chemicals were of analytical grade.

Methods

Preparation of Multiple Emulsions

Multiple emulsions were prepared by two step emulsification process. First primary emulsion is formulated in a high stirring speed. Then the so formed primary emulsion is again emulsified to get multiple emulsions.[3]

Step 1 (Preparation of Primary Emulsion)

• 45ml of distilled water containing 50mg of drug was gradually added to 55ml of oil phase containing primary emulsifier span80(4ml) and 25mg of drug with continuous stirring at 5000rpm for 5min.

• Primary emulsion(w/o) was formulated.

STEP 2 (Secondary Emulsification)

• 20ml viscous primary emulsion was emulsified further with an external aqueous phase containing secondary emulsifier (Tween 80) and 25mg drug with continous stirring at 1000rpm for 10 minutes.

• Water / oil/ water (w/o/w) multiple emulsion was formed.

Formulation of multiple emulsions with optimized primary emulsion

Eight batches (F1-F8) of primary emulsions was prepared by placing the drug concentration as constant (100mg) whereas, the rpm, concentration of surfactant & ratio of aqueous and oil phase had taken as variables. The particle size & drug release from the emulsion was kept as the response factors. By using the design expert (stat ease) software, the optimization profiles were obtained. The optimized formula had used for the preparation of multiple emulsion of Atorvastatin with good particle size & drug release profile. Suitable flavouring agents were added to obtain palatability to multiple emulsion. Variables for optimization are given in table 1 and formula for optimum batch of primary emulsion is given in table 2.

Table 1: Variables for optimization

	Variables	+1	-1
X1	RPM	5000	3000
X2	Surfactant concentration	4ml	12ml
X3	Ratio of aqueous & oil phase	30:70	45:55

Preparation of calibration curve

10mg of AT dissolved in sufficient quantity of methanol and volume was made up to 100ml with methanol. From the stock solution, different dilutions from 2-20μg/ml was prepared with the same diluting medium to obtain a calibration curve. Absorbances of solution were spectrophotometrically determined at 247.5nm.

Microscopic Analysis

Microscopic analysis was carried out using an research microscope (labomed LX 300) combined with a computer imaging system, and observations were made at 40 X magnification after diluting in the appropriate amount of external phase of the emulsion. The shape and homogeneity of the multiple droplets is followed immediately after the preparation of the multiple emulsion formulations. A picture of the multiple emulsion formulation was taken.

Droplet Size Analysis

The mean droplet size of the multiple emulsion formulations was determined using particle size analyzer (Malvern Mastersizer) for the freshly prepared formulation.

pH

pH of the freshly formulated emulsion was done using digital pH meter. Here the digital pH meter is calibrated to neutral pH by dipping the glass electrode end in freshly prepared distilled water for several minutes. Then the glass electrode is dipped in the emulsion and pH reading was noted.

Entrapment efficiency

Entrapment efficiency was determined by taking freshly prepared W/O/W multiple emulsions and immediately centrifuged at 4000 r/min for 10 min. Then 1ml of the aqueous phase (the lower layer) was precisely withdrawn through 2 ml hypodermic syringe and diluted properly with 0.1N HCl. The solution was filtered with a whatman filter paper and drug content was analyzed on UV spectrophotometer at 240 nm. The Encapsulation Efficiency was determined by following equation:

% EE = [(Total drug incorporated –Free Drug)/ Total drug] X 100

Invitro drug release study

The *in vitro* drug release study was carried out on a simple dissolution cell using cellophane membrane. Prior to release studies, the cellophane membrane was soaked in distilled water for 6 hours, washed frequently 4 times by changing distilled water, then immersed in 5% v/v glycerol solution for at least 60 min and washed finally with 5 portions of distilled water. Freshly prepared multiple emulsion (15ml) was added to donor chamber, made up of a hollow glass tube (2.5 cm in diameter and 10 cm in length) and membrane was tied on bottom end of the tube with a nylon string. This tube was dipped into

1000 ml vessel containing 900 ml of PBS pH 6.8 and was stirred at 75 rpm on a magnetic stirrer and maintained at 37 °C which acted as receiving chamber. Aliquots of 1ml were collected from receiving chamber at predetermined time intervals and the drug contents determined on UV spectrophotometer at 240 nm after suitable dilution.[5]

Determination of Drug Release Mechanism[6,7]

In order to understand the mechanism of drug release, Invitro drug release data were treated to kinetic models such as zero order, first order and Higuchi model and korsmeyerpeppa"s model.

Ex Vivo Release study

Drug release study was performed on the rat ileum by following perfusion method. The special apparatus consist of U shaped glass tube having 1 cm inner diameter with cannulated cut on the upper half arm of the U tube.[8]

Viscosity study

Viscosity is a principal parameter when any flow measurements of fluids, such as liquids, semisolids. Gases and even solids are made. Brookfield deals with the liquids and semisolids. Brookfield viscosity usually refers to a viscosity measurement performed with a Brookfield Viscometer, sometimes referred to as a Brookfield viscosimeter. There are several models of viscometer available from Brookfield but the majority operates in the same manner: the viscometer motor rotates the spindle at a defined speed (measured in rpm) or shear rate and the viscometer measures the resistance to rotation and reports a viscosity value.[9]

Stability study of optimized formulation

Formulations were subjected to stability studies for a period of 30 days. The samples were withdrawn after 30 days and were evaluated pH, entrapment efficiency and release profile.

RESULTS AND DISCUSSION

The Drug (Atorvastatin) powder was examined for its organoleptic properties like colour, and odour and it is observed that the Atorvastatin was white amorphous powder. The sample was quantitatively tested for its solubility in various solvents. Solubility study in different solvents revealed that it is freely soluble in methanol, slightly soluble in alcohol and insoluble in distilled water. The melting point of Atorvastatin Calcium was found to be 159.2-160.7.

Preparation of calibration curve

Absorbances of solution were spectrophotometrically determined at 247.5nm. A standard calibration curve for the drug was obtained by measuring absorbance at 247.5nm and by plotting the graph of absorbance Vs concentration.
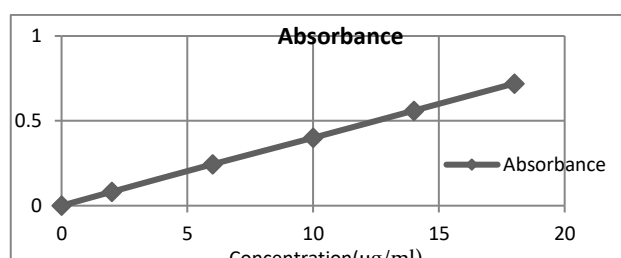

Figure 1: Calibration curve of Atorvastatin

Evaluation of Multiple Emulsions

Microscopic Analysis

Microscopic analysis was carried out using an research microscope (labomed LX 300), and observations were made at 40 X magnification after diluting in the appropriate amount of external phase of the emulsion the image taken via microscope.
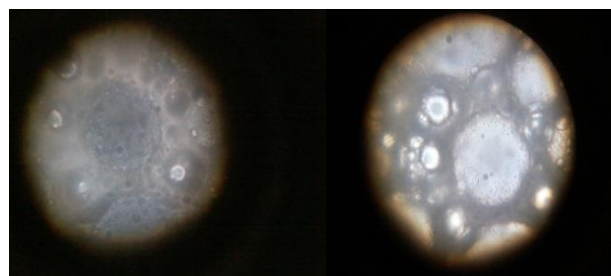

Figure 2: Microscopic images of multiple emulsions

Table 2: Formula for optimum batch of primary emulsion

Number	Rpm	Conc. of surfactants (ml)	Ratio of aqueous& oil phase(ml)	Particle size (µm)	Drug release (%)
1	3000	4	30:70	5.26	84.13

Table 3: Mean droplet size of emulsion

Sl. No	1	2	3	4	5	6	7	8	9	10
Size(µm)	12.7	14.7	16.4	11.2	10.3	11.3	5.5	5.4	6.4	7.5

Table 4: Cumulative Drug Release of Atorvastatin Multiple emulsion (Exvivo)

Time	0	15	30	45	60	120	180	240	300
%CDR	0	5.3	10.0	15.5	29.1	38.5	50.6	76.5	88.9

Droplet Size Analysis

The mean droplet size of the multiple emulsion formulation was determined using stage micrometer and eyepiece micrometer for the freshly prepared formulation. The mean droplet size of multiple emulsions was 7.8µm. The obtained droplet sizes are given in table3.

pH

pH of the freshly formulated emulsion was done using pH paper and it was found to be 8.

Entrapment efficiency

After formulating Atorvastatin multiple emulsions, the drug content was estimated by UV spectrophotometer at λ max 247.5nm. The Entrapment efficiency of multiple emulsions was 88.5%.
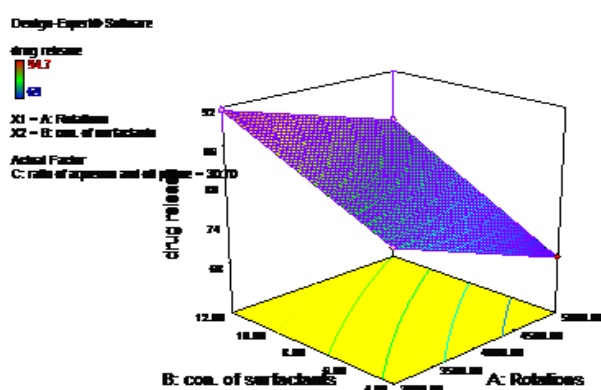

Figure 3: 3D plot of drug release

In-vitro drug release studies

In- vitro drug release studies were carried out in PBS of pH 7.4 as the dissolution medium. Studies were performed as per the procedure described in methodology.
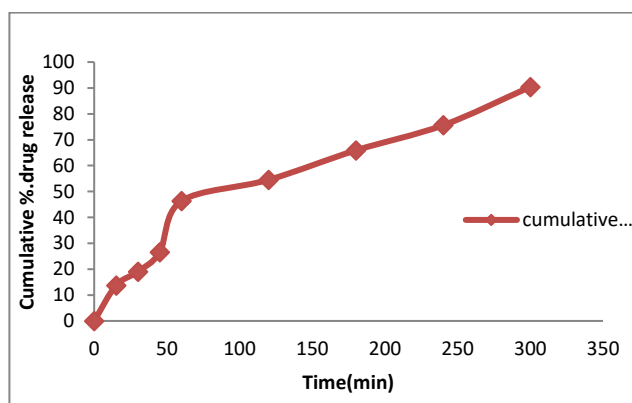

Figure 4: Invitro Cumulative drug release.

Ex vivo drug release study

Exvivo release studies were carried out by using the U shaped apparatus. The rat ileum was tied in this apparatus and multiple emulsion was filled in it. The Multiple emulsions was filled in the ileum and placed inside the dissolution vessel with pH 7.4phosphate buffer. The drug released in the media was measured. The cumulative drug release at various time intervals are mentioned in table4.

Viscosity

Viscosity of ME was determined by using the Brookfield viscometer by selecting the spindle number and rpm. The viscosity of the formulation at different rpm has performed and is given in table 5.

Table 5: Viscosity of the formulation

rpm	Spindle Number	Viscosity(Cp)	Torque%
100	18	16	53.5
50	18	22	36.6
60	18	7	14

Kinetic Modeling

The results obtained of *in vitro* release studies were attempted to fit into various mathematical models. From the results given in table 6, it is clear that the drug release shows first order kinetics for the formulation.

Table 6: Model fitting for the release profile of Atorvastatin Multiple Emulsion

Formulation	Correlation coefficient(r)value			
	Zero order	First order	Higuchi	Korsmeyer Peppas
ME	0.823	0.967	0.957	0.952

Stability study

Prepared formulation was subjected to stability study for 30days. Sample was withdrawn after 30 days and was evaluated for parameters like particle size, pH and *in vitro* drug release. Formulation showed slight decrease in entrapment after 30 days of storage. The in vitro drug release from the formulation was also decreased after stability study period. This may be due to decrease in the relative drug content.

Comparative study of multiple emulsion of Atorvastatin with marketed Atorvastatin tablets

The Multiple emulsions was evaluated for in vitro dissolution study and compared with marketed tablet under same experimental conditions and the data was recorded as a chart in figures.
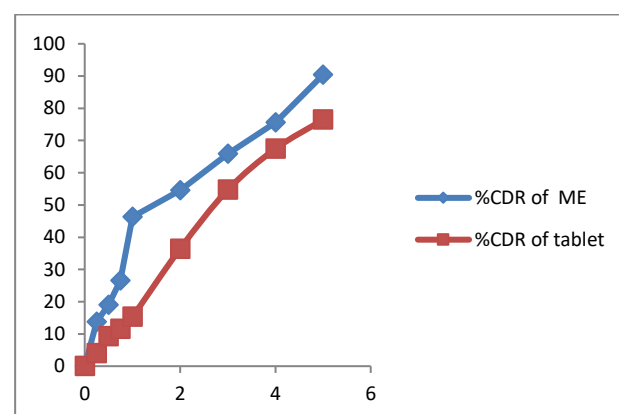

Figure 5: Comparative study of %CDR of ME & tablet of Atorvastatin

CONCLUSION

The study was aimed at the Formulation, Optimization and evaluation of multiple emulsion of Atorvastatin. Two different non-ionic surfactants were used for the formulation. Atorvastatin, a synthetic HMG Co-A Reductase inhibitor, is widely used in treatment of primary hypercholesterolemia and Dyslipidaemia. ME was prepared by two step emulsification process. The main purpose was to develop stable multiple emulsion with higher solubility & drug release. The study revealed that multiple emulsions can be optimized for good stability and higher drug release by optimizing different formulation variables like type & proportion of primary & secondary emulsifier and phase volume ratio of internal phase: external phase; and process variables like speed & time of stirring during primary & secondary emulsification.

This study concludes that Multiple Emulsion formulation can provide consistent and prolonged release of Atorvastatin. It will lead to sustained action of the entrapped drug that reduce the side effects associated with frequent administration of the drug and potentiate the therapeutic effects of the drug.

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