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FORMULATION AND EVALUATION OF NOVEL MICROEMULSIONS OF ATORVASTATIN CALCIUM TRIHYDRATE

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Abstract

The objective of the present study was to develop a novel microemulsion drug delivery system of a poorly water soluble drug Atorvastatin calcium trihydrate. The oral delivery of such drugs is frequently associated with implications of low bio availability, high intra and inters subject variability and lack of dose proportionality. Phase solubility studies were conducted for the maximum solubility of Atorvastatin. Highest solubility was found in Tween80, Transcutol (surfactants), PEG400, Cremophor RH40 (cosurfactant) and Isopropyl myristate, Sunflower oil (oil). Ternary phase diagrams were constructed to evaluate microemulsion regions. FTIR analysis was done for investigating the drug-excipient interactions. The mean globule size of both microemulsion was observed to be below 200nm for the optimized formulations and the zeta potential was negative. The dissolution of emulsion formulations was compared to commercial tablets, the results indicated that the rate of dissolution of developing formulations containing Atorvastatin was increased compared with that of commercial tablets. SEM studies were done for the shape and morphology of the globules. Thus, micoemulsions can be regarded as novel and commercially feasible alternative to the current Atorvastatin formulations.

Keywords: Tween80, Transcutol, Cremophor RH40, Ternary phase diagrams, Zetapotential.

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Introduction

Oral ingestion is the most convenient and commonly employed route of drug delivery due to its ease of administration, high patient compliance, cost-effectiveness, least sterility constraints and flexibility in the design of the dosage form. The oral bio-availability depends on several factors including aqueous solubility, drug permeability, dissolution rate, first pass metabolism, pre-systemic metabolism and susceptibility to efflux mechanisms. The most frequent causes of low oral bio-availability is attributed to poor solubility and low permeability.[1] Nearly 40% of new drug candidates exhibit low solubility in water, which leads to poor oral bio-availability, high intra and inter-subject variability, and lack of dose proportionality. Thus, for those drugs, the adsorption rate from the lumen of the gastro intestinal tract is controlled by dissolution. Hence, producing suitable formulations is essential to improve the solubility and bioavailability of such drugs.[2]Conventional techniques such as salt formation micronization and solubilization using cosolvents, use of permeation enhances[3],oily solutions and surfactant dispersions[4] were

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previously employed to increase the oral bio-availability have shown limited utility. Although recently developed strategies, such as solid dispersion technology[5] and inclusion complexes employed cyclodextrins[6], exhibit good potential, they are successful in some instance and are specific to drug candidates. Over the past few decades colloidal systems have been explored as potential delivery systems, because of their compartmentalized hydrophobic and hydrophilic domains, where both polar and non-polar molecules could be encapsulated and stabilized. The dispersed systems studied for the delivery of different kinds of drugs are liposome, noisome, nanoemulsions, organogel, lipid dispersions, polymeric micelle and cationic polymer.[7-10]

Microemulsions are extensively studied and recently, much attention has been focused on surfactant and lipid based formulations to improve the oral bioavailability of poorly soluble drugs. Microemulsions are successfully designed to deliver hydrophobic drug and shown to increase their oral absorption and bio-availability. Microemulsions are thermodynamically stable, optically transparent, isotropic dispersions of aqueous and hydrocarbon liquids stabilized by an interfacial film of surfactant molecules.[11-15]

The basic components that are used in the designing of microemulsions are oil, surfactant, co surfactant, water and drug. When a microemulsion is formulated, the drug is initially loaded into oil and/ or surfactant phases. When introduced into an aqueous phase, the drug will subsequently partially distribute into the aqueous medium. However, if sufficient water is not present, microemulsions may not form. In addition, some drug molecules are amphiphilic to some degree, and can potentially act as co-surfactants and/or hydrophilic agents. Thus, the drug may affect the formation and properties of formed microemulsions.[16-22]

Materials and methods

Materials

Atorvastatin calcium trihydrate Gift sample from Bright labs, Hyderabad. Span 20, Tween 20, Tween 80, Isopropyl myristate from Sd- Fine Chemicals limited, Mumbai. All reagents and ingredients were of analytical grade.

Methods

Formulation design of microemulsion containing Atorvastatin

A series of micro emulsions were prepared in each of five formulations with varying ratio of oil, surfactant, and cosurfactant. In all the formulations, the amount of drug was constant (10mg/ml). Briefly, drug was dissolved by co-surfactant (PEG400) in glass vials. Oils and surfactant were accurately weighed and incorporated into glass vials, then water is added and components were mixed by gentle stirring and vortex mixing and heated at 37˚C in incubator, until Atorvastatin perfectly has dissolved. The mixture was stored at room temperature until used.

Table No. 01: Formulation of Atorvastatin microemulsions

Formulation	% w/w of different components in formulation			Quantity of drug loaded (mg/ml)
	Oil	S mix	Water	
MA1	6.9	76.7	16.2	10
MA2	8.6	78.2	13	10
MA3	3.2	67.7	29	10
MA4	5.3	74.6	20	10
MA5	**2.3**	**65.1**	**32.5**	**10**

Characterization

Drug excipient compatibility studies

FTIR spectrums of Atorvastatin and drug-microemulsion formulation were obtained by means of a FTIR spectrophotometer (bruker-alpha T).The samples were prepared by the potassium bromide disk method and measurements were attempted with the accumulations of 20 scans and a resolution of 4 cm^{-1} over the range of 400-4000cm^{-1}.After running the spectra, significant peaks realating to major functional groups were identified; spectra of the subsequent sample of the same compound were compared with the original.

Droplet size measurement

The mean droplet size of emulsion globules was determined by using photon correlation spectroscopy (which analyses the fluctuations in light scattering due to Brownian motion of the particles) using nano zeta sizer able to measure

sizes between 10-3000nm.Light scattering was monitored at 25˚C at a 90˚ angle. The dispersed formulations were measured after dilution (1:100) to produce the required count rate (50-200) to enable the accurate measurement.

Zeta potential

The zeta potential of microemulsion was determined using nano zeta sizer. The Charge on emulsion droplets and their mean zeta potential values were obtained from the instruments.

Viscosity determination

The viscosity of the microemulsion formulation had generally been very low. This was expected, because one of the characteristics of microemulsion formulation is lower viscosity. The viscosity of formulations was determined without dilution using BROOKFIELD-DV-11 + pro viscometer using spindle00 UV adapter at 25±0.5˚C.

Conductivity determination

A conduct meter (lab India pico +) was used in non-linear temperature compensation mode, according to EN 27888 conductivity was determined between 45 and 90˚C under magnetic stirring at an agitation of 250rpm.This temperature ranges permit a steady state to be achieved, either as an emulsion o/w(high steady state) or as an emulsion w/o (low steady state) in different conditions tested. The recording of conductivity relative to temperature permits the determination of phase inversion temperature. Conductivity values lower than 10 micro cm^{-1} means that the continuous phase is oil, where as a higher steady state shows that water is the continuous phase.

Drug content

A measured quantity of microemulsion was added to 100ml of pH 6.8 buffer. The resulting mixture was kept at 24hrs in a dark place. Then the solution was filteres through membrane filter of 0.45µm pore size and 1ml of this solution was diltuted to 10ml using 6.8pH buffer. After further dilutions with mobile phase, the samples were analysed by UV spectrophotometer for drug content at 230nm. The drug content was determined using the relationship.

$$\frac{experimental\ drug\ content}{theoretical\ drug\ content} \times 100 = Drug\ content$$

Transmission electron microscopic studies

Examining the surface of a polymeric drug delivery system can provide vital information on the porosity and microstructure of these systems. The distribution and morphology of the surface and the encapsulated matrix can also be directly observed. The most common technique used for characterizing the surface morphology of drug delivery systems is transmission electron microscopy (TEM). The sample sizes, which can be analysed using this method, range from nanometers to micrometers to centimeters.

Invitro dissolution studies

The release of Atorvastatin from the microemulsion formulations was determined according to USP dissolution apparatus type II. To permit the quantitative drug release from microemulsion formulation, 900ml of pH 6.8 buffer and 900ml of 0.1N HCL was placed in the dissolution vessel and then the microemulsion formulation filled ion hard gelatin capsule was placed in the dissolution medium and was agitated at 50rpm at 37˚C. At pre-determined time intervals of 5,10,,20,30,40,50 and 60 minutes, 5 ml of the samples were withdrawn and the drug concentration was determined by UV spectrophotometer at wavelength 230nm.The volume withdrawn was replaced each time with the fresh dissolution medium. Cumulated released amounts were plotted as a function of time.

Stability studies

The microemulsion formulations were put into empty hard gelatin capsules (size0) and subjected to stability studies 25˚C and 60% relative humidity (RH), 30˚C/65%RH and 45˚C /75%RH. Samples were charged in stability chambers with humidity and temperature control. They were withdrawn at specified intervals for analysis over a period of 3 months for intermediate and accelerated conditions and 6 months for long-term conditions. Drug content of the capsules was analyzed using a previously developed and validated stability indicating UV method.

Results and discussion

FTIR Studies

From the FTIR data it was evident that the drug and excipients doses not have any interactions**.** Hence, they were compatible.

Droplet size measurement

Droplet size measurement results were shown in Table.2.

Table No. 02: Droplet size measurement of microemulsions

Formulation code	Droplet size (nm)
MA1	221.7 ±0.5
MA2	236.4 ±1.25
MA3	210 ±0.95
MA4	213.1 ±0.86
MA5	207 ±1.35

Zeta potential

Zeta potential is used to decide the electrophoretic mobility of particles. The scale of the zeta potential gives the suggestion of the probable stability of the colloidal system. The zeta potential was found to be -1.2 &-0.1 mV.

Table No. 03: Zeta potential of optimized formulations

Formulation code	Zeta potential in mV
MA3	-1.2
MA5	-0.1

Viscosity and Conductivity determination

Viscosity and conductivity of MA3 & MA5 are 24.8±0.62, 20.235±0.5 and 21.8±0.2, 22.554±0.5.

Table No. 04: Viscosity and Conductivity of optimized formulations

Formulation	Mean viscosity (cP)	Mean conductivity ($s.m^{-1}$)
MA1	23.1 ±0.36	17.035± 0.5
MA2	20.525 ±0.5	32.011 ± 0.5
MA3	24.8 ±0.62	20.235± 0.5
MA4	22.5 ±0.8	16.035 ± 0.5
MA5	21.8 ±0.2	22.554± 0.5

TEM and SEM

Scanning electron microscopic (SEM) & Transmission electron microscopic photographs showed nanospheres of spherical, discrete nature, and distinct size and a nearly smooth surface.

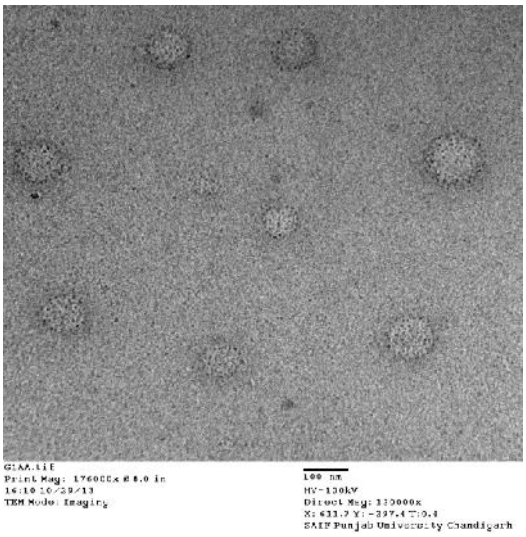

Fig. No. 01: (A) TEM of microemulsions

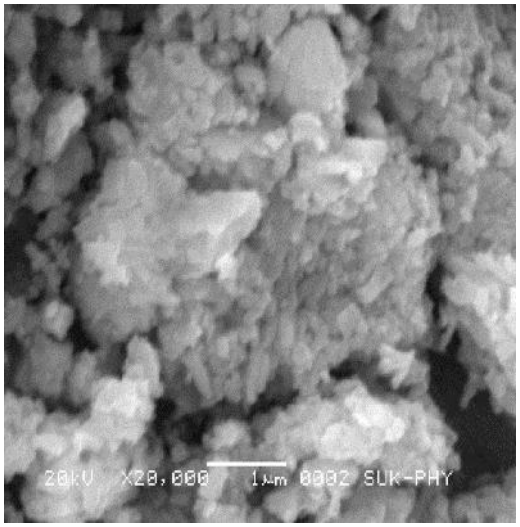

Fig. No. 01: (B) SEM of microemulsions

Invitro dissolution studies

The MA3 showed drug release of 95.3±0.9% for a period 40 mins and MA5 showed drug release of 99±0.4% for a period 40 mins.

Table No. 05: Invitro dissolution studies of microemulsions

Time (min)	MA1	MA2	MA3	MA4	MA5	Tablet
5	9.45 ±0.05	10.35 ±0.2	22.5 ±0.1	27 ±0.2	**31.5 ±0.5**	9.9 ±0.6
10	19.33 ±0.2	20.46 ±0.4	35.64 ±0.3	54 ±0.4	**54 ±0.7**	13.5 ±0.8
20	44.35 ±0.5	46.82 ±0.6	64.29 ±0.5	49.5 ±0.6	**49.5 ±0.9**	24.6 ±0.1
30	71.22 ±0.6	72.32 ±0.8	78.06 ±0.7	81 ±0.8	**81 ±0.2**	39.8 ±0.3
40	81.22 ±0.8	82.77 ±0.1	95.3 ±0.9	83 ±0.1	**99 ±0.4**	47.2 ±0.5
50	90.12 ±0.1	90.91 ±0.3	-	85.5 ±0.3	**-**	-
%Drug released(10mg)	9.012	9.091	9.53	8.55	**9.9**	4.72

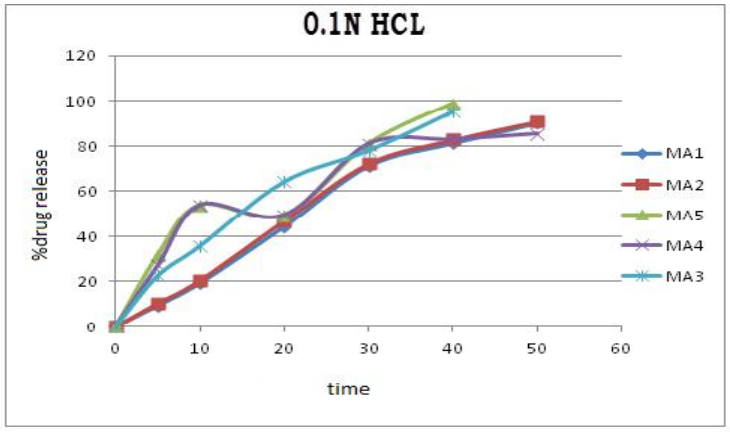

Fig. No. 02: % drug release of microemulsions

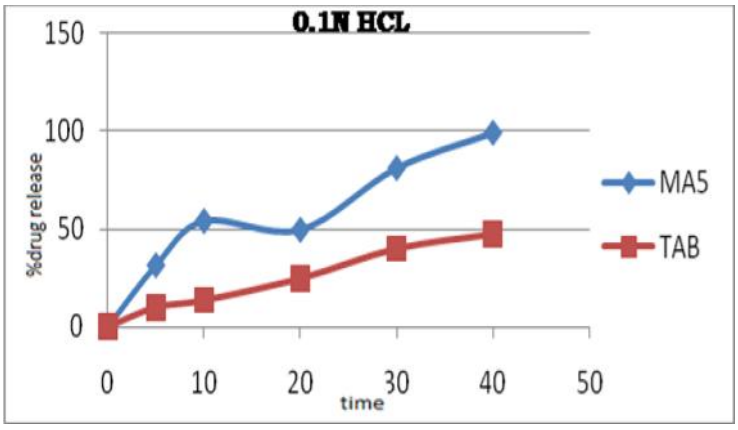

Fig. No. 03: % drug release of microemulsions & conventional marked tablet

Stability studies

Table No. 06: Stability studies of microemulsions

Formulation code	Sampling point	Droplet size(nm)	% drug content
MA3	0 days	207.4 ±0.1	99.67 ±0.2
	45 days	212.3 ±0.5	98.65 ±0.8
	3 months	200.5 ±0.3	97.87 ±0.09
MA5	0 days	210 ±0.5	99.33 ±0.05
	45 days	203.8 ±0.4	98.77 ±0.6
	3 months	201 ±0.1	96.10 ±0.01

Conclusion

Novel emulsion formulations i.e. microemulsions are a promising approach for the formulation of Atorvastatin. The oral delivery of hydrophobic drugs can be made possible by Microemulsions which have been shown to substantially improve oral bioavailability with a future development of this technology. These novel emulsions will continue to enable novel applications in drug delivery and solve problems associated with the delivery of poorly soluble drugs. micoemulsions can be regarded as novel and commercially feasible alternative to the current Atorvastatin formulations.

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