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Formulation and Evaluation of a Novel Microemulsions of Atorvastatin Calcium Trihydrate

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A B S T R A C T

The objective of the present study was to prepare and evaluate Hydrogel Beads for the controlled release of Nizatidine from the prepared Hydrogel beads using different polymers. The Hydrogel beads were prepared by ionotropic gelation method. The prepared Hydrogel beads were characterized for FTIR, scanning electron microscopy (SEM), the percentage drug content, entrapment efficiency, *in vitro* dissolution studies, Release order kinetics, Stability studies. The Particle size of hydrogel beads was determined with the help of SEM and it was found to be ranging from 500-650 µm. The swelling Index of Nizatidine containing Hydrogel beads F 11 formulation contains the value 28 ±1 %. The FT-IR and DSC study confirmed that no chemical interaction took place during encapsulation process. Entrapment efficiency was in range of all formulation 76.2-77.3%. The drug entrapment of various batches varied from 47.35% to 99.68%. The F_{11} formulation observed a zero order release based on the regression coefficient value in kinetics study.

Keywords: PEG 400, Cremophor RH40, Ternary phase diagrams, Zeta potential.

A R T I C L E I N F O

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1. Introduction

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. 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 [1]. Microemulsions are thermodynamically stable, optically transparent, isotropic dispersions of aqueous and hydrocarbon liquids stabilized by an interfacial film of surfactant molecules [2,3]. Atorvastatin is an anticholesteremic agent, hydroxymethyl glutaryl-CoA reductase inhibitor. However, the low solubility and poor dissolution of this drug affects its rate of absorption, resulting in a low and variable oral bioavailability [4,5]. Hence it becomes necessary to develop Atorvastatin novel emulsion formulations with enhanced solubility and bioavailability

2. Material and methods

Materials:

Atorvastatin was gift sample from Bright labs Hyderabad, Transcutol was a generous gift sample from Gattefosse France, Cremophore RH 40 was a gift sample from BASF corp Germany, Tween 80, sunflower oil and PEG 400 was purchased from S.D. fine chemicals ltd, Mumbai.

Methods:

Solubility study of Atorvastatin in various excipients:

The solubility of Atorvastatin in various oil, surfactant, co-surfactant was determined. Solubility studies were conducted by placing an excess amount of drug in each vehicle in a 2ml Microtube (Axygen MCT 200) containing 1.5ml of the vehicle. Then the mixture was vortexed and kept for 48hrs at 25˚C in a Orbital shaking incubator (Remi electrotechnik ltd.) to facilitate the solubilization [6]. The samples were centrifuged at 3000rpm for 15min to remove the undissolved drug. The supernatant was taken and the concentration of drug in each vehicle were quantified by UV-spectrophotometer.

Construction of Pseudo-ternary phase diagrams:

The pseudo-ternary phase diagrams of oil, surfactant co surfactant and water were developed using surfactant titration method: The mixtures of oil and water at certain weight ratios were titrated with surfactant: co surfactant mix in a drop wise manner [7]. Five types of surfactant phases were prepared: Tween 80+ PEG 400(1:1, 1:2, 2:1, 1:3, 3:1) . For each phase diagram at a specific ratio of surfactant/co surfactant transparent and homogenous mixture of oil and water was formed under the mixing by cyclomixer. Then, visually observed for phase clarity and flow ability [8,9]. After the identification of micro emulsion region in the phase diagrams, the micro-emulsion formulations were selected at desired component ratios, in order to form the micro emulsion.

Formulation design of microemulsion containing Atorvastatin:

A series of micro emulsions were prepared in each of five formulations with varying ratio of oil, surfactant, cosurfactant. In all the formulations, the amount of drug was constant (10mg/ml). Briefly, drug was dissolved by co-surfactant (PEG400) in glass vials [10]. 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 [11]. The mixture was stored at room temperature until used.

Table 1: Formulation table of Atorvastatin microemulsions

F	% w/w of different components in formulation			Quantity of drug loaded (mg/ml)
	Oil	Smix	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**

Drug excipient compatibility studies:

FTIR spectrums of Atorvastatin and drug-microemulsion formulation were obtained by means of a FTIR spectrophotometer (bruker-alpha T) [12]. 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 [13]. 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 were determined using nano zetasizer. Charge on emulsion droplets and their mean zeta potential values (± SD) were obtained from the instruments [14].

Viscosity determination:

The viscosity of microemulsion formulation generally was very low. This was expected, because one of the characteristics of microemulsion formulation is lower viscosity[15]. The viscosity of formulations was determined without dilution using Brookfield-DV-11 + pro viscometer using spindle 00 UV adapter at 25±0.5˚C.

Conductivity determination:

A conductometer (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 [16]. 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 were added to 100ml of pH 6.8 buffer. The resulting mixture was kept at 24hrs at 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 [17]. After further dilutions with mobile phase, the samples were analyzed by UV spectrophotometer for drug content at 230nm.

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) [18]. The sample sizes, which can be analysed using this method, range from nanometers to micrometers to centimeters.

Thermodynamic stability studies:

Freeze thaw cycle:

Freeze thawing was employed to evaluate the stability of the formulations. The formulations were subjected to 3-4 freeze thaw cycles, which included freezing at -4˚C for 48 hours followed by thawing at 40˚C for 48 hrs. Centrifugation was performs at 3000rpm for 5 min [19]. The formulations were then observed for phase separation [19]. Only formulations that were stable to phase separation were selected for further studies.

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.

In-vitro 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 [20]. 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 fresh dissolution medium. Cumulated released amounts were plotted as a function of time.

3. Results and Discussion

The solubility of Atorvastatin in various vehicles is presented. Tween80 and PEG400 provided higher solubility than other vehicles and sunflower oil was selected, for the optimal novel emulsion formulation resulting in improved drug loading capabilities. The phase study revealed that the maximum proportion of oil was incorporated in microemulsion systems when the surfactant/cosurfactant ratio was 1:2.

The dissolution studies for all the stable microemulsion formulations were determined in USP dissolution medium pH 6.8. at the end of one hour, the dissolution of the MA3 microemulsion formulation was significantly greater than that of other formulations. It suggests that Atorvastatin dissolved perfectly in microemulsion formulation, and could be released due to its small droplet size which permits a faster rate of drug release into the aqueous phase. The formulation was found to be stable for 3months; there was no significant change in the drug content, or particle size of the resultant emulsion. It was also seen that the formulation was compatible with the hard gelatin capsule shells, as there was no sign of capsule shell deformation. Furthermore, the formulation was found to show no phase separation, drug precipitation, or capsule leaks. Thus, these studies confirmed the stability of the developed formulations.

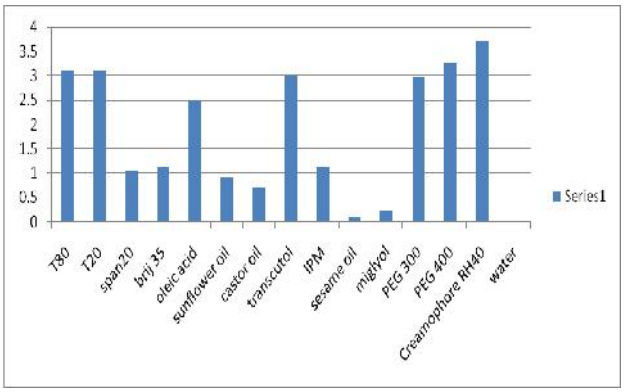

Figure 1: Solubility of Atorvastatin in various oils, surfactants, cosurfactants.

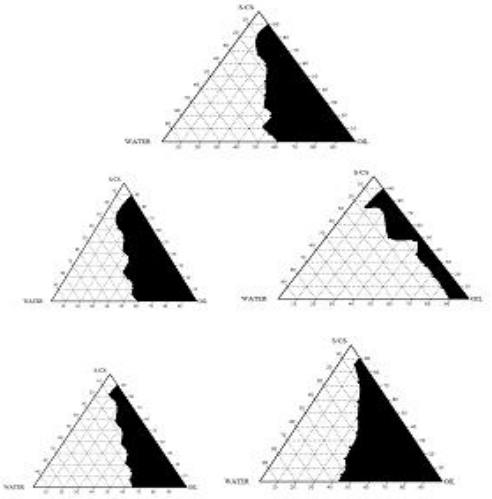

Figure 2: Ternary Phase Diagrams

a) Pseudo ternary phase diagram indicating the efficient microemulsion region containing (Tween80/PEG400) = (a) 1:1 (w/w), 1:2, 2:1,1:3,3:1

Drug excipient compatibility studies

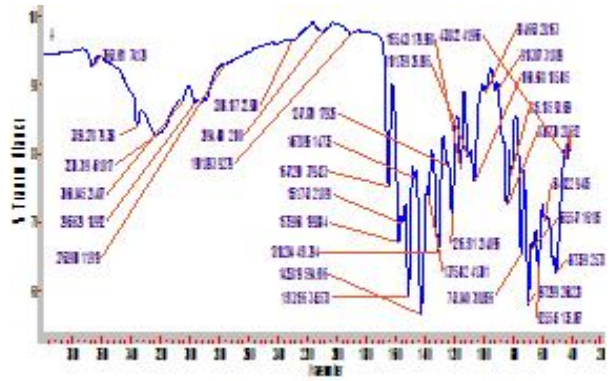

Figure 3a: Atorvastatin calcium trihydrate

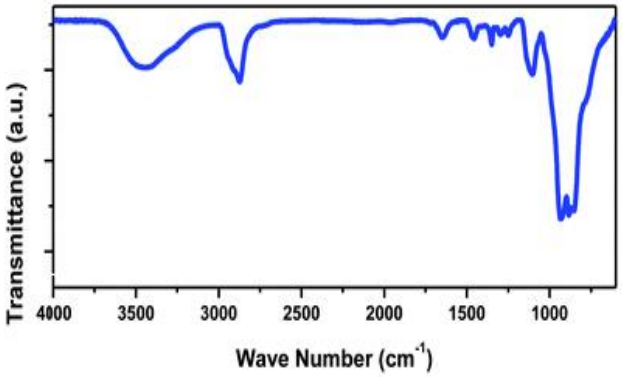

Figure 3b: PEG 400Tween 80 Microemulsion formulations

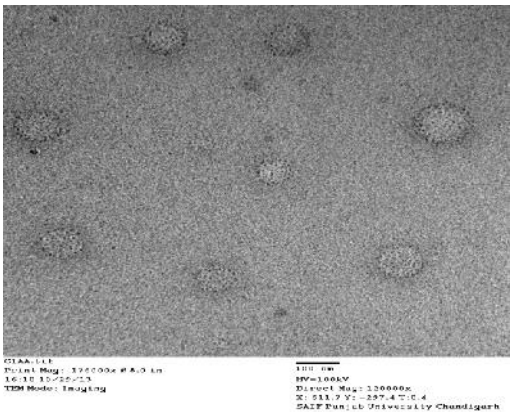

Figure 4a: TEM

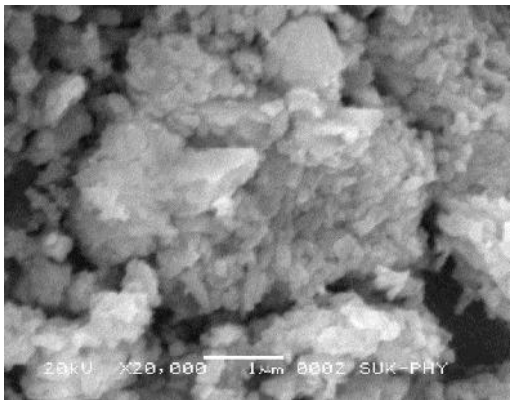

Figure 4b: SEM

Table 2: Phase separation and Visibility grade

Formulation code	Visibility grade	Phase separation	Precipitation
MA1	I	×	××
MA2	I	×	××
MA3	I	×	××
MA4	I	+	××
MA5	I	×	××

Table 3: Droplet size measurement

Formulation code	Droplet size (nm)	Zeta potential in mV
MA1	221.7 ±0.5	-1.2
MA2	236.4 ±1.25	-0.1
MA3	210 ±0.95	-0.4
MA4	213.1 ±0.86	-1.5
MA5	207 ±1.35	-1.3

Table 4: Viscosity and Conductivity

Formulation	Mean viscosity (cP)	Mean conductivity ($s.m^{-1}$)	Drug content
MA1	23.1 ±0.36	17.035± 0.5	97.18 ±0.05
MA2	20.525 ±0.5	32.011 ± 0.5	98.67 ±0.06
MA3	24.8 ±0.62	20.235± 0.5	99.33 ±0.3
MA4	22.5 ±0.8	16.035 ± 0.5	97.82 ±0.5
MA5	21.8 ±0.2	22.554± 0.5	99.53 ±0.2

Table 5: Stability Studies

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

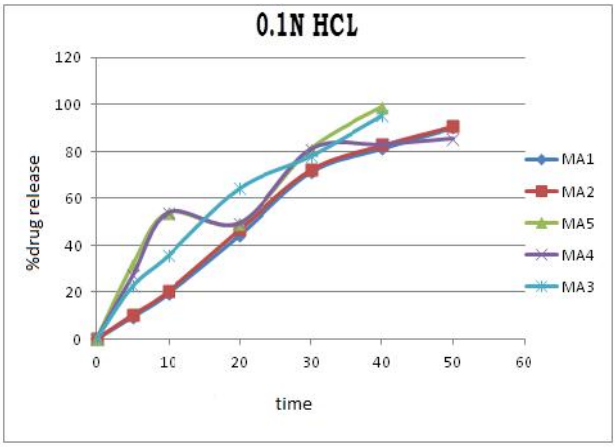

Figure 5: Dissolution of microemulsions in 0.1N HCL

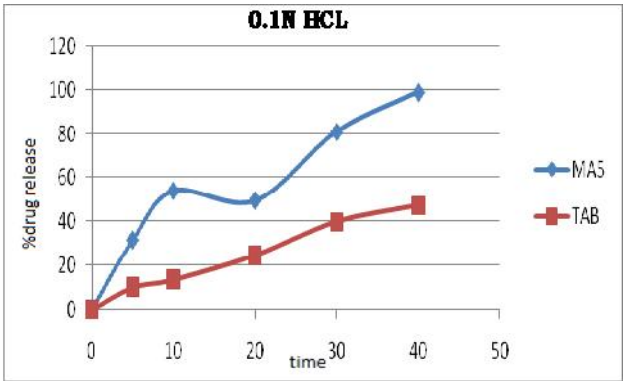

Figure 6: Dissolution of selected microemulsion and conventional marked tablet of Atorvastatin in 0.1N HCL

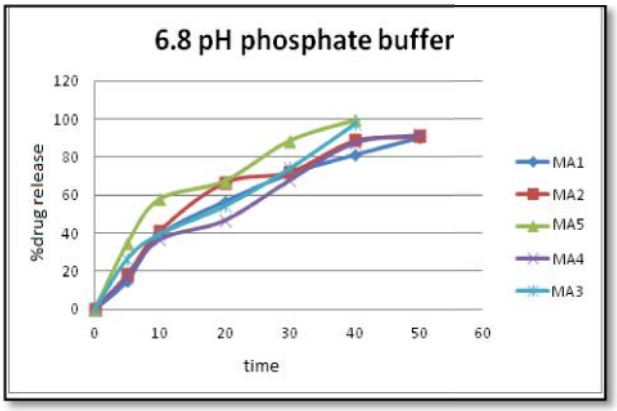

Figure 7: Dissolution of microemulsions in 6.8 pH phosphate buffer.

4. 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 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.

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