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DEVELOPMENT OF MICROEMULSION FOR SOLUBILITY ENHANCEMENT OF ATORVASTATIN CALCIUM

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ABSTRACT: Atorvastatin Calcium, competitive inhibitor of hydroxymethylglutaryl-coenzyme A (HMG-CoA) reductase, the rate-determining enzyme in cholesterol biosynthesis via the mevalonate pathway, thereby lowering cholesterol level in plasma of body. Oral bioavailability of Atorvastatin Calcium is very low (less than 4%), due to its poor water solubility. The aim of this investigation was to develop a microemulsion of Atorvastatin Calcium for enhancing its solubility, and its oral bioavailability. For this purpose, initially, solubility of Atorvastatin Calcium was determined in various vehicles. Oil, Surfactant and Cosurfactant were selected based on the solubility and HLB value. Pseudo-ternary phase diagrams were constructed to identify the microemulsion existing zone. Solubility study was also performed for optimization of formulation. The optimized microemulsion formulation was characterized for its % transmission, globule size, zeta potential, conductivity, % assay, and phase separation study. Globule size and zeta potential of the optimized microemulsion formulation were found to be 57.61 nm, and -19.4 mV, respectively. The viscosity and conductivity data indicated that the microemulsion was of the o/w type. Solubility of Atorvastatin Calcium was successfully enhanced via Isopropyl myristate microemulsion, compared with distilled water (pH = 7.4). 82.32% and 79.74 % of the drug content were found to be released within 8 h in the *in-vitro* and *ex-vivo* studies, respectively. Hence, by formulating into microemulsion, the solubility of Atorvastatin Calcium was found to be significantly enhanced.

INTRODUCTION: Numbers of the new drug candidates have poor water solubility, and thus oral delivery is frequently associated with low bioavailability of drugs.

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Microemulsions have been emerged as potential drug delivery vehicle, due to their simplicity of preparation, transparency and ability to be filtered and incorporate a wide range of drugs of varying solubility. Oil-in-water (o/w) microemulsion is the most suitable formulation, which increases the solubility of drugs by dissolving drugs with low water solubility into an oil phase. They can also enhance oral bioavailability by reducing the globule size (< 100 nm), and hence increase the rate of absorption due to surfactant-induced permeability changes [2].

Atorvastatin Calcium selectively and competitively inhibits the hepatic enzyme HMG-CoA reductase. As HMG-CoA reductase is responsible for converting HMG-CoA to mevalonate in the cholesterol biosynthesis pathway, these results in a subsequent decrease in hepatic cholesterol levels [3]. Decreased hepatic cholesterol levels stimulate upregulation of hepatic LDL-C receptors which increases hepatic uptake of LDL-C and reduces serum LDL-C concentrations.

However, oral bioavailability of Atorvastatin Calcium is very low (less than 4%), due to poor water solubility.

Hence, the objective of this study was to enhance the solubility of Atorvastatin Calcium by formulating it into a microemulsion.

MATERIALS AND METHODS:

Materials: Atorvastatin Calcium was received as a gift sample from Torrent Pharmaceutical Ltd. (Gujarat, India). Capmul CMC, *Accenon CC*, Labrafil, Cremophor RH 40 and Transcutol P were received from Abitec Corporation (USA) as gift sample. Iso-propyl myristate, Labrafac PG, Soya bean oil, Tween-60, Tween-80, was purchased from Ozone internationals (Mumbai, India). Isopropyl alcohol, Isobutyl Alcohol, PEG 400 and PEG 600 were purchased from S.D. Fine Chemicals (Mumbai, India). All the other chemicals and solvents were of analytical reagent grade and used without further purification.

Selection of the components: Selection criteria for oil phase and surfactant for SMEDD are drug solubility, biocompatibility and HLB value i.e., 12-18. Selection of the oil phase was based upon the maximum solubility of the drug [4]. Different oils including Capmul CMC, Iso-propyl myristate, Labrafac PG, and Soya bean oil were taken for solubility studies. Several surfactants including Tween-80, Tween-60, *Accenon CC*, Cremophor RH 40 and Labrafil were screened. Co-surfactants were selected based on their capability to form stable microemulsion with relevant surfactants at a minimum concentration.

Based on this, several co-surfactants including Isopropyl alcohol, Isobutyl Alcohol, PEG 400, PEG 600 and Transcutol P were screened.

Pseudo-ternary phase diagram: Pseudo-ternary phase diagrams were constructed to obtain the suitable components, and their concentration ranges that resulted in a large existence area of microemulsion were preferred [5]. In order to optimize the concentration of oil phase, surfactant and co-surfactant, different batches of varied concentration were prepared and titrated with distilled water till transparency persisted. Ternary phase diagram was prepared by using a constant ratio of surfactant to co-surfactant. Four ratios of surfactant (*Accenon CC*) and co-surfactant (Transcutol P) were selected (1:1, 2:1, 3:1 and 4:1).

Preparation of Microemulsion: Predetermined amounts of the drug were dissolved in the required quantity of oil. Surfactant and co-surfactant were added to the above mixture as a fixed ratio. Distilled water was added gradually with continuous stirring, which resulted in the formulation of a transparent and homogenous microemulsion [6]. Store the clear transparent oily liquid mixture at room temperature until further use.

Characterization of Microemulsion:

1. **Transmittance test:** % Transmittance of the optimized SMEDD and its diluted formulations was determined by measuring transmittance at 650 nm with a UV spectrophotometer (UV-1601-220X, Shimadzu).

2. **Globule size and Zeta Potential measurements:** The globule size and zeta potential of the microemulsion was determined by dynamic light scattering, using a Zetasizer HSA 3000 (Malvern Instruments Ltd., Malvern, UK).

3. **Electrical conductivity:** To a mixture of oil, surfactant and co-surfactant, water phase was added drop wise and the electrical conductivity of optimized formulation was measured using a conductometer (CM 180 conductivity meter, Elico, India) at ambient temperature and at a constant frequency of 1 Hz.

4. **Drug stability:** The optimized microemulsion was stored under cold condition (4-8^{o}C), room temperature and at elevated temperature (50 ±

2 ^{o}C). After every 2 months the microemulsion was analyzed for phase separation, % transmittance, globule size and % assay [7].

5. **Drug solubility:** Excess amount of drug was added to the optimized microemulsion formulation as well as each individual ingredient of the formulation. After continuous stirring for 72 h at room temperature, samples were withdrawn and centrifuged at 5000 rpm for 10 min. The amount of soluble drug in the optimized formulation as well as each individual ingredient of the formulation was calculated by subtracting the drug present in the sediment from the total amount of drug added [8]. Comparison of solubility of drug in microemulsion with respect to its individual ingredients was performed.

(VI) % Assay: The optimized microemulsion was diluted with 0.1N HCl and was stored for 2 hrs. Then both the samples were analyzed by UV spectrophotometer at 247 nm

Drug release studies:

1. ***In-vitro* drug release:** The diffusion studies were carried out, using modified dissolution apparatus was filled with 200ml of phosphate buffer (pH=6.8) equilibrated at 37±2°C. Developed formulation (equivalent to 10 mg of Atorvastatin Calcium) and plain drug solution was placed in dialysis beg separately. Dialysis bag was put in the dissolution medium stirred at 50 rpm. 5 ml Aliquots were withdrawn at the interval of 1 hr and were analyzed spectrophotometrically at 247 nm [9].

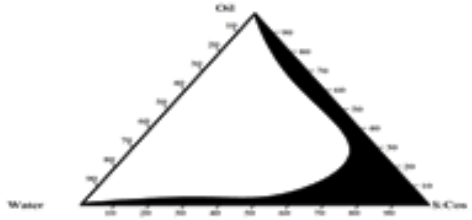
2. ***Ex-vivo* drug release:** *Ex-vivo* drug release into phosphate buffer pH 6.8 was studied using rat ileum within modified dissolution apparatus at 37±2°C.

   Microemulsion formulation (equivalent to 10 mg) and the plain drug solution was put into the cavity of rat ileum and both ends were tied, ileum was put in the dissolution medium stirred at 50 rpm [10]. The amount of drug released from the microemulsion formulation was estimated spectrophotometrically at 247 nm, by withdrawing samples at predetermined time intervals.

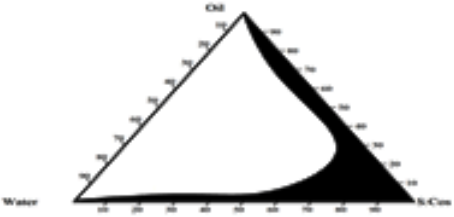
RESULTS AND DISCUSSION:

Preparation of Microemulsion: The maximum amount of drug was found to be dissolved in Iso-propyl myristate (49.0 ± 1.27 mg/mL).Therefore, this oil was selected for microemulsion formulation. The selection of surfactant was mainly based on required HLB value to form o/w microemulsion which should be between 12-18. Solubility of drug, as well as Iso-propyl myristate was higher in *Accenon CC*. Co-surfactants were selected based on their capability to form a stable microemulsion with the relevant surfactants at a minimum concentration. Transcutol P was selected for *Accenon CC* containing microemulsions.

Pseudo-ternary phase diagrams were constructed to obtain the appropriate components and their concentration ranges that can result in a large microemulsion existence area. From the ternary phase diagrams shown in **Figure 1**, it was concluded that the highest microemulsion zone was achieved for the microemulsions containing *Accenon CC*/Transcutol P at a ratio of 2:1. A maximum amount of water uptake was found at 1.5% Iso-propyl myristate and 45% *Accenon CC*/Transcutol P (2:1) concentration.



SURFACTANT: CO-SURFACTANT = 1:1



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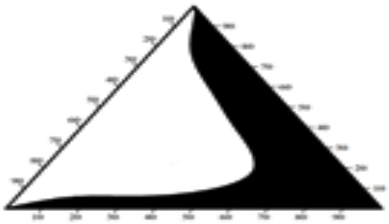


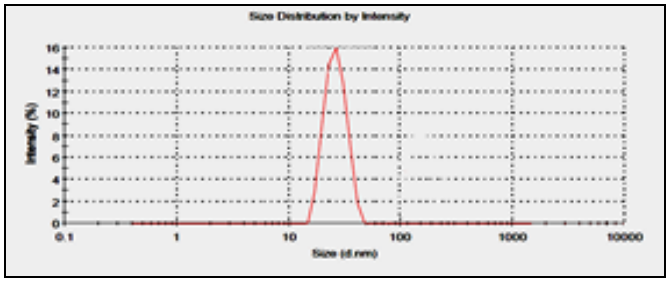
SURFACTANT: CO-SURFACTANT = 3:1

FIGURE 1: PSEUDOTERNARY PHASE DIAGRAMS OF MICROEMULSIONS

Characterization of Microemulsions:

1. **Transmittance test:** The percentage of transmittance of the optimized microemulsion formulation, as well as its 100 times dilution with 0.1N HCl, was checked at 650 nm and found to be 99.68 ± 0.041 and 99.89 ± 0.028, respectively.

2. **Globule size measurement:** The optimized Iso-propyl myristate microemulsion showed very small particle size (*i.e.* 57.61 nm) and upon 100 times dilution with 0.1N HCl and storage for 3 h, it showed very little change in particle size (*i.e.* 60.90 nm). The value of polydispersity index (PI) of both samples were found to be below 1.0, suggesting that upon dilution with gastric fluid in body, the optimized microemulsion formulation remains stable and will not convert into macroemulsion [11]. Results of globule size have been shown in **Figure 2**.



A

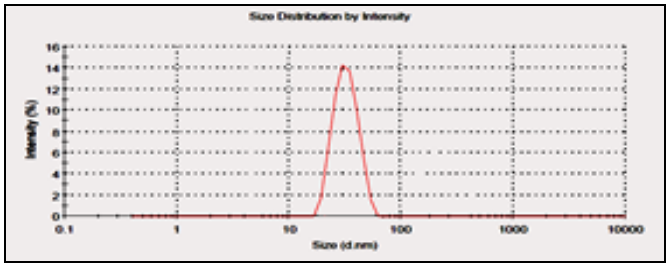


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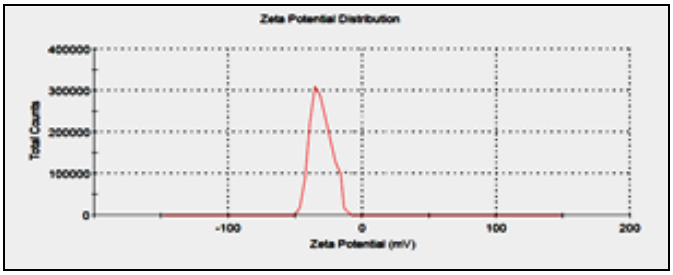
FIGURE 2: AVERAGE GLOBULE SIZE OF THE OPTIMIZED MICROEMULSION (A) AND ITS DILUTED FORM (100 TIMES WITH 0.1N HCl) (B)

3. **Zeta potential measurements:** Zeta potential results of the optimized microemulsion and its diluted form (100 times diluted with 0.1N HCl) have been shown in Figure 3, and were found to be -19.4 mV and -14.4 mV, respectively. Due to the slightly negative charge of the globules of microemulsion, Aggregation is not expected to take place [12].



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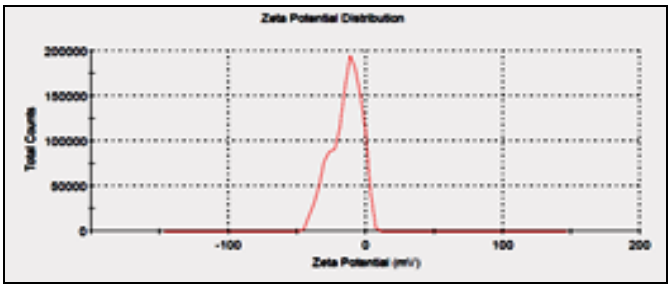

B

FIGURE 3: ZETA POTENTIAL OF THE OPTIMIZED MICROEMULSION (A) AND ITS 100 TIMES DILUTED FORM (B)

4. **Electro-conductivity measurement:** As long as the percentage of aqueous phase was less than 7, the electrical conductivity (σ) was almost zero. During the aqueous phase titration, electrical conductivity increased rapidly up to 38% water content. When the percentage content of the aqueous phase was above 38%, the conductivity of the system was not affected significantly with further addition of the aqueous phase.

   Hence, the middle phase microemulsion was kept between 7% to 38% aqueous phase addition. Above 38% aqueous phase addition, it is converted to an o/w microemulsion [13]. Since the optimized Iso-propyl myristate microemulsion formulation contained 75% of the aqueous phase, hence, the structure of the optimized Iso-propyl myristate microemulsion is an o/w structure.

5. **Drug solubility:** Solubility of the drug in microemulsion formulation and the individual ingredients of the microemulsion have been shown in **Figure 4**. The results indicate an enhanced solubility of Atorvastatin Calcium in the optimized formulation, when microemulsion was compared to its respective individual ingredients.

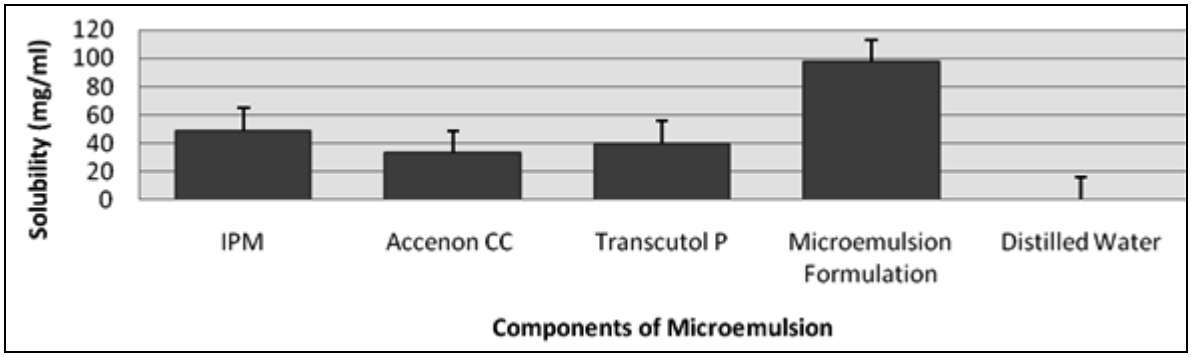

FIGURE 4: SOLUBILITY STUDY OF ATORVASTATIN CALCIUM IN DIFFERENT COMPONENTS OF MICROEMULSION AND THE OPTIMIZED MICROEMULSION (N = 3)

6. **Stability studies:** Results of temperature stability studies on the optimized microemulsion has been given in **Table 1**. The optimized microemulsion was also subjected to centrifugal stability studies. Results obtained from both the stability studies indicated that the optimized Iso-propyl myristate based microemulsion was stable up to 6 months.

Table 1: Effect of temperature on stability of the optimized microemulsion formulation (n = 3)

Temperature (°C)	Phase Separation		% Transmittance		Globule size (nm)		% of Assay	
	After 3 month	After 6 month	After 3 month	After 6 month	After 3 month	After 6 month	After 3 month	After 6 month
Cold temperature (2 -8 °C)	No	No	97.5±1.2	95.4± 1.3	65.2 ± 1.6	71.5± 1.3	92.2 ± 0.3	88.4 ± 0.2
Room temperature (25±2 °C)	No	No	99.6±1.4	98.9± 1.6	57.6 ± 1.4	60.5 ± 1.5	98.9 ± 0.8	94.5 ± 0.6
Elevated temperature (50 ± 2 °C)	No	No	98.4±1.5	94.6± 0.7	67.6 ± 0.9	79.3 ± 1.2	87.5 ± 0.9	81.3 ± 0.4

7. **% Assay:** Drug content of optimized microemulsion formulation was measured by U.V. Spectrophotometer at 247 nm and was found to be 98.93±0.89 %.

8. ***In-vitro* drug release studies:** Release profile of Atorvastatin Calcium was carried out from the optimized microemulsion formulation, as well as the plain drug solution, and the results have been shown in **Figure 5**. After 8 h, drug released from the plain solution and the Iso-propyl myristate microemulsion was 67.21% and 82.32%, respectively.

   From this study, it can be concluded that the extent of diffusion of Atorvastatin Calcium from the microemulsion formulation is greater than the plain drug solution. The Iso-propyl myristate microemulsion showed a faster Atorvastatin Calcium release, than the plain solution, due to small globule size and the partitioning of drug within the oil phase. The sustained drug release observed may be due to the practically insoluble nature of Atorvastatin Calcium in water [14].

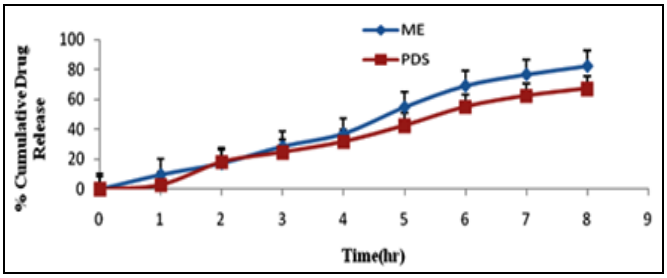

FIGURE 5: *IN-VITRO* DRUG RELEASE PROFILE OF ATORVASTATIN CALCIUM FROM THE MICROEMULSION FORMULATION AND THE PLAIN DRUG SOLUTION (n = 3)

9. ***Ex-vivo* release studies:** *Ex-vivo* release profile of Atorvastatin Calcium was carried out on the optimized microemulsion formulation, as well as the plain drug solution and the results have been shown in **Figure 6**. After 8 h, the amount of drug released from the plain solution and the microemulsion

formulation was 64.21% and 79.74%, respectively. From this study, it can be concluded that the extent of diffusion of Atorvastatin Calcium from the Iso-propyl myristate microemulsion is greater than the plain solution, due to the penetration enhancing effect of surfactant and co-surfactant present within the microemulsion formulation, aiding passage of the drug molecule through the intestinal membrane and small globule size of the microemulsion formulation [15].

Overall, the microemulsion formulation showed a higher extent of absorption than the plain drug solution. The sustained profile of drug release observed may be due to the fact that drug is present within the oil phase and hence has a higher partition coefficient.[16]

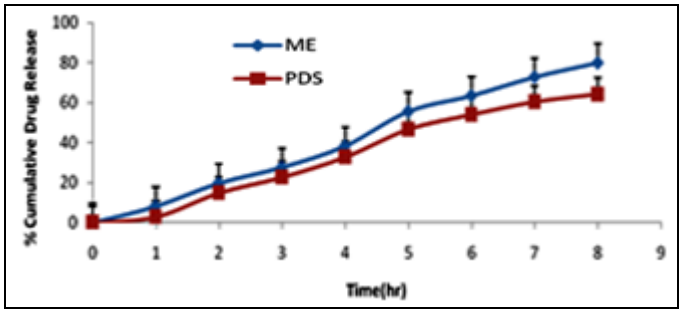

FIGURE 6: *EX-VIVO* RELEASE PROFILE OF ATORVASTATIN CALCIUM FROM THE MICROEMULSION FORMULATION AND THE PLAIN DRUG-CONTAINING SOLUTION (n = 3).

CONCLUSION: This study demonstrates that the microemulsion formulation can be employed to enhance the solubility of poorly water soluble drugs like Atorvastatin Calcium. The optimized microemulsion formulation containing Iso-propyl myristate (1.5%), *Accenon CC* (18%), Transcutol P (4%) and distilled water is a transparent and low viscosity system, with a globule size of 57.61 nm. The stability studies confirmed that the optimized microemulsion was stable for six months.

Results from the *in-vivo* studies revealed that the developed microemulsion formulation possessed a higher rate and extent of absorption, compared to the plain drug solution. The solubility profile of drug indicated that the microemulsion formulation can enhance solubility compared to distilled water (pH = 7.4), which may increase the oral bioavailability of Atorvastatin Calcium. However, further studies on animals and human being are needed to be performed before this formulation can be commercially exploited.

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