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RESEARCH ARTICLE

Formulation and *In-vitro* Evaluation of Self Micro-Emulsifying Drug Delivery System of Atorvastatin

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ABSTRACT

The oral delivery of lipophilic drugs presents a major challenge due to low aqueous solubility of such compounds. Atorvastatin (ATV), a 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitor, is a plasma lipid-regulating agent. The chief intention of this work is to develop an orally stable self Micro-emulsifying drug delivery system by evaluating its *in vitro* potential. Components of SMEDDS were assessed by solubility studies on various oils, surfactants, co-surfactant. Ternary phase diagrams were constructed to identify area of micro-emulsification for the selected systems. Characterization of SMEDDS was done by Physical method, Droplet size, Zeta potential determination, drug loading capacity, Transmission test, Cloud point measurement and *in vitro* release study. The optimal formulation consisted of mixture of Drug (0.99%), Acrysol K150 and PEG 400 (1:1) and Gelucire 44/14 (19.80%). Droplet size of optimized batch was 87.65nm with PdI 0.493. Drug loading capacity was 2-3 times than the Actual dose of ATV. Transmission values were above 99% in pH 1.2, pH 6.8 and distilled water. Cloud point of formulations was about74°C. *In vitro* release inspection of optimal formulation illustrated a complete release of Atorvastatin from SMEDDS within 20 min. Our study concludes that the SMEDDS shows potential approach for the poorly water soluble drugs including Atorvastatin.

KEYWORDS

SMEDDS, Atorvastatin, Phase titration method, Droplet size, Zeta potential

INTRODUCTION

The oral route has been the major route of drug delivery for chronic treatment of many diseases. Oral drug delivery system is the most cost-effective and leads the world wide drug delivery market. However, in the present scenario, oral drug delivery is continuously looking into newer avenues as 40% of new drug candidates have poor water solubility and/or absorption, high intra-and inter-subject variability, rapid metabolism, high fluctuation in the drug plasma level, variability due to food effect, and lack of dose proportionality which are playing major role in disappointing *in vivo* results leading to failure of conventional drug delivery system[1,2]. Lipid-based formulations are of good interest in recent years. Lipid carriers such as oils, surfactant, emulsions, self-emulsifying formulations, self

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nanoemulsifying systems, self-microemulsifying systems[3] are having potential to engulf lipophilic drug and most importantly remain inside until reaches to blood circulation. Self microemulsifying systems (SMS) are mixtures oil, surfactants, co-surfactants that form fine o/w microemulsion when introduced into aqueous phases under gentle agitation.[4] Various formulation strategies to improve the dissolution and bioavailability have been subjected for BCS Class II drug but amongst them self microemulsifying drug delivery systems (SMEDDS) have established particular interest as a means of enhancingoral bioavailability of poorly absorbed drugs.[5] SMEDDS spread into fine emulsion droplets inside the gut lumen where drug remains in solution state, evading the dissolution step that intermittently limits the rate of absorption of hydrophobic drugs from the crystalline state.

Atorvastatin is a competitive inhibitor of HMG-CoA reductase. Unlike most others, however, it is a completely synthetic compound. HMG-CoA reductase catalyzes the reduction of 3-hydroxy-3-methylglutaryl-coenzyme A(HMG-CoA) to mevalonate, which is the rate-limiting step in hepatic cholesterol biosynthesis. Inhibition of the enzyme decreases cholesterol synthesis, increasing expression of low-density lipoprotein receptors (LDL receptors) on hepatocytes. This increases LDL uptake by the hepatocytes, decreasing the amount of LDL-cholesterol in the blood. Like other statins, atorvastatin also reduces blood levels of triglycerides and slightly increases levels of HDL-cholesterol.

Present study shows preparation of various kind of SMEDDS formulation by performing trial batches of various oils (Gelucire 44/14, Capryol 90, Oleic acid, Arachis oil), surfactants (Acrysol derivatives and Labrafil M 2125), co-surfactants (PEG400) with drug to assess the ability of drug in solubilized form and to form a transparent solution for better emulsification ratio of Surfactant: Cosurfactant: Cosolvents remains same. Non ionic surfactants with high HLB (HLB = 10) and subsequent hydrophilicity is necessary for the instant creation of oil in water droplets and/or rapid spreading of the formulation in the aqueous environment providing a good dispersing/self-emulsifying performance. The surfactants are amphiphilic in nature have ability to dissolve and solubilize to some extent high quantities of the hydrophobic drug. In SMEDDS generally surfactant of HLB value 8-16 is used.

The prime objective of the investigation is to formulate, optimize and stabilize SMEDDS containing Atorvastatin with suitable surfactant and co-surfactant. To achieve High drug loading capacity with ability to retain drug in solubilized form. Solubility of drug plays a very important role in dissolution and hence absorption of drug which hypothetically affects its bioavailability.

MATERIAL AND METHODS

Atorvastatin was received as a gift sample from Healthy life Pharma (Mumbai, India) Gelucire44/14, Labrafil M 2125, Capryol 90 were gift samples from Gattefosse (Mumbai, India), Acrysol Derivatives (K140, K150, K160, EL135) were generous gift from Corel Pharma (Ahmedabad, India), PEG400, Methanol were purchased from S. D. fine chemicals (Mumbai, India) Double distilled water was prepared freshly whenever required. Various oils are purchased as received. All other chemicals were of analytical grade.

Solubility Studies

The solubility of Atorvastatin in various oils, surfactants, and co-surfactant was determined. Briefly, an excess amount of Atorvastatin (approximately 100 mg) was introduced into 1ml of each vehicle, and mixture was kept in 20 ml beaker (covered with Aluminum foil).The mixture was stirred using mini magnetic stirrer (DBK Instruments) for up to the saturation point in beaker at 37°C. Samples were kept aside and allow standing for 48hr at ambient temperature to attain equilibrium. The equilibrated sample was centrifuged at 2,000 rpm for 10 min to remove the un-dissolved drug. Aliquot of 0.2ml was taken from clear supernatant. Filtered using membrane filters (0.45µm). The concentration of Atorvastatin was then quantified using U.V spectrophotometer (Systronics 2201). Table 1

shows the solubility studies.

Table 1: Solubility of Atorvastatin in Various Lipid Excipients

Excipient	Solubility (mg/ml)
Oils	
Gelucire 44/14	101.17±1.24
Capryol 90	52.17±2.92
Oleic acid	54.51±1.82
Arachis oil	57.84±3.12
Surfactants	
Labrafil M 2125	101.17±1.61
Acrysol K 160	52.17±2.46
Acrysol K 150	82.61±1.37
Acrysol K 140	49.82±2.31
Acrysol EL135	70.79±2.16
Co-surfactant/Co-solvent	
PEG 400	40.15±3.56

Construction of Pseudo Ternary Phase Diagram Study[6]

On the basis of the trial batches of excipients, the pseudoternary phase diagrams of oil (Gelucire 44/14), surfactant: co-surfactant (Acrysol K 150: PEG 400), and distilled water were developed using water titration method. The mixture of oil and surfactant/co-surfactant

(Smix) at certain weight ratio were diluted with water in drop wise manner. For each phase diagrams at specific ratio of surfactant/co-surfactant (1:1, 1:2, 1:3, and 2:13:1) and oil ratio (1:9, 1:8.5, 1:8, 1:7.5, 1:7, 1:6.5, 1:6,1:5.5, 1:5, 1:4.5, 1:4, 1:3.5, 1:3, 1:2.5, 1:2, 1:1.5, 1:1, 1.5:1and 2:1) was taken and prepared transparent and homogeneous mixture by mini magnetic stirring. Then, each mixture was titrated with water and visually observed for phase clarity and flow ability. After the identification of microemulsion region in the phase diagrams, the microemulsion formulations were selected at desired component ratios. The physical state of the microemulsion was marked on a pseudo-three-component phase diagram with one axis representing aqueous phase, the other representing oil and the third representing a mixture of surfactant and co-surfactant at fixed weight ratios (Smix ratio). Figure 1 shows phase diagrams of various ratios.

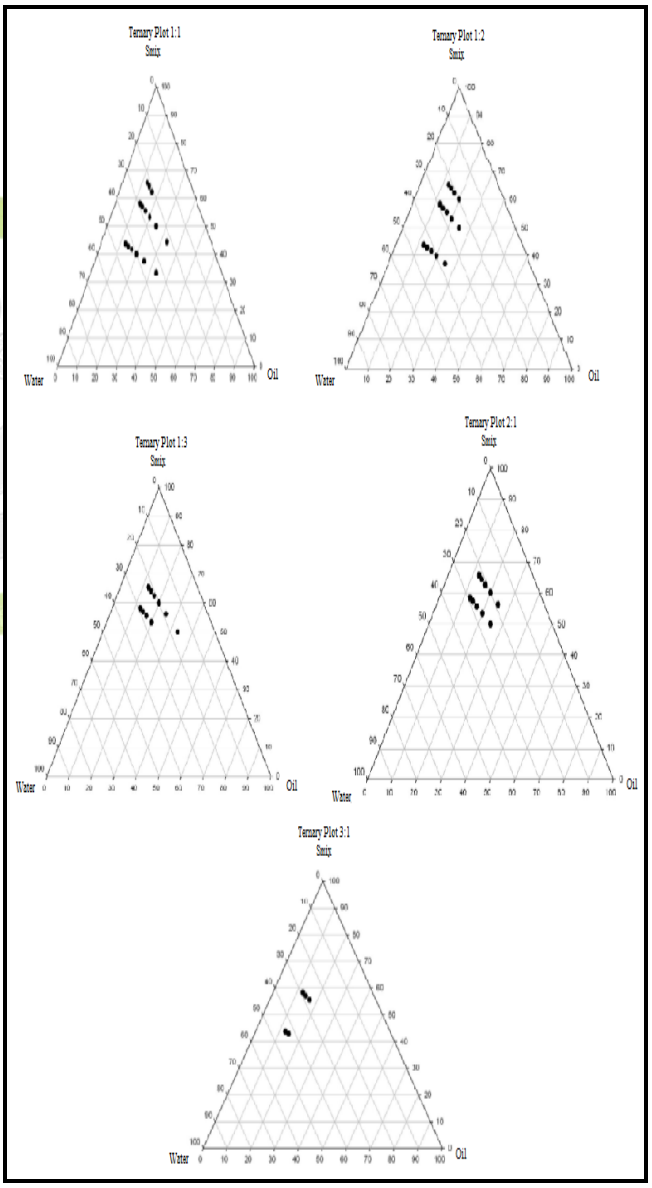

Figure 1: Ternary Phase Diagram Study on Atorvastatin SMEDDS

Table 2: Different Batches for Optimization

Ingredient	F1 Qty. (mg)	F2 Qty. (mg)	F3 Qty. (mg)	F4 Qty. (mg)	F5 Qty. (mg)	F6 Qty. (mg)	F7 Qty. (mg)	F8 Qty. (mg)
Drug : ATV	10	10	10	10	10	10	10	10
Gelucire 44/14	200	100	200	100	200	100	100	100
Acrysol K 150	400	450	540	600	600	680	220	300
PEG 400	400	450	260	300	200	220	660	600
Total	1010	1010	1010	1010	1010	1010	1010	1010

Drug Loading Capacity

This study was done with the purpose to achieve the highest drug loading capacity in the formulation specifically for high dose drug molecules. Make 1 ml SMEDDS formulation as per 1:1 and 8:2 S_{mix}: oil (In 3 beaker). In all three beakers add 10 mg, 20mg and 30 mg drug respectively and after completely dissolve of the drug add slowly water in it (up to 100 ml) and cover it with aluminium foil. Keep it for 24 hrs to check the re-precipitation of drug.

Figure 2: Bach of Formulations F1 to F8

Preparation of Liquid SMEDDS Formulation

- Mix accurate quantity of Acrysol K 150 and PEG 400 in a beaker thoroughly.
- Accurately weighed quantity of Atorvastatin was added to above mixture.
- Mixed properly on mixture then given amount of Gelucire 44/14 was added. Mixture was stirred thoroughly to get clear transparent liquid.

Dilution Study

Dilution may better mimic conditions in the stomach following oral administration of SMEDDS pre-concentrate. Dilution study was done to access the effect of dilution on SMEDDS pre-concentrates. An entire 10 mg of Atorvastatin integrated in SMEDDS formulation.1 part SMEDDS of each solution was diluted with 10 parts of distilled water, Phosphate buffer pH 1.2 and Phosphate buffer pH 6.8 and observed. Observation of dilution studies is shown in Table 3.

SMEDDS Characterization

Thermodynamic Stability Studies[7]

- ✓ **Heating cooling cycle:** Six cycles between refrigerator temperature4°C and 40°C with storage at each temperature of not less than 48 h was studied. Stable formulations at these temperatures were subjected to centrifugation test.
- ✓ **Centrifugation:** Accepted formulations were centrifuged at 5000 rpm for 30 min. Those formulations having absence of any phase separation were taken for the freeze thaw stress test.

- ✓ **Freeze thaw cycle:** Three freeze thaw cycles amongst -21 °C and +25 °C with storage at each temperature for not less than 48 h was done for the formulations.

Formulations which passed these thermodynamic stress tests were further taken for the dispersibility test for assessing the efficiency of self-emulsification.

Dispersibility Test

The efficiency of self-emulsification of oral microemulsion was assessed using a standard USP dissolution apparatus type II.[7] 1 ml of each formulation was added to 500 ml of water at 37 ± 0.5°C. A standard stainless steel dissolution paddle rotating at 50 rpm provided gentle agitation. The *in vitro* performance of the formulations was visually assessed using the following grading system:

Grade A: Rapidly forming (within 1 min) microemulsion, having a clear or bluish appearance.

Grade B: Rapidly forming, slightly less clear emulsion, having a bluish white appearance.

Grade C: Fine milky emulsion that will form within 2 min.

Grade D: Dull, grayish white emulsion having slightly oily appearance that is slow to emulsify (longer than 2 min).

Grade E: Formulation, exhibiting either poor or minimal emulsification with large oil globules present on the surface.

Percentage Transmittance (λ max 650 nm)

Stability of ATV SMEDDS formulations on dilution was checked by measuring Transmittance through U.V. Spectrophotometer.

Table 3: Thermodynamic Stability and Dispersibility Test of Different Formulations

Code	Effect of Temperature on Phase Separation, Flocculation, Precipitation						Dispersibility study	Inferen -ce
	After 4 week		After 8 week		After 12week			
	2-8˚C	Room Temperature	2-8˚C	Room Temperature	2-8˚C	Room Temperature		
F1-F8	Not seen	Not seen	Not seen	Not seen	Not seen	Not seen	Grade A	Pass
	Centrifugation stability data (Phase separation)							
	After 1 month		After 2 month		After 3 month			
F1-F8	Not seen		Not seen		Not seen		Grade A	Pass
	Heating and cooling cycle(Creaming or Cracking)							
	After 12 hrs		After 24 hrs		After 48 hrs			
F1-F8	Not seen		Not seen		Not seen		Grade A	Pass
	Freeze thaw cycle (Phase Separation)							
	At -12˚C		At 5˚C		At 25˚C			
F1-F8	Not seen	Not seen	Not seen	Grade A	Pass	F1-F8	Not seen	Not seen

% Transmittance of samples was measured at 650nm and for each sample three replicate measurement was performed. In this study SMEDDS formulations were diluted 100 times with distilled water, phosphate 1.2 and phosphate buffer pH 6.8.[8]

Cloud Point Measurement Study

The cloud point is a crucial feature in the SMEDDS consisting of non-ionic surfactants, and it is responsible for the successful formation of a stable microemulsion. The SMEDDS were compared for cloud point value. Each formulation was diluted with distilled water in the ratio of1:250 and placed in a water bath with gradual increase in temperature. The point at which cloudiness occur was noted as cloud point.[9-11]

Determination of Droplet Size/Distribution and Zeta Potential

Atorvastatin SMEDDS formulations (approximately 1ml) was diluted with purified water (100mL) and gently shaken in a volumetric flask at 25°C. The droplet size/distribution and zeta-potential were analyzed by dynamic light scattering technique using a Zetasizer (Nano ZS, Malvern Instruments, UK) equipped with a 4.0mW He-Ne red laser (633nm).

In vitro Drug Diffusion Studies[12]

The *in vitro* diffusion studies were carried on these out by using the dialysis technique (A. Paradakar et al 2007) One end of pretreated cellulose dialysis tubing (7 cm in length) was tied with thread and 0.5 ml of self-emulsifying formulation (equivalent to 10 mg Atorvastatin) was placed in it. Activate dialysis bag by soaking (one day before dissolution study) in the phosphate buffer pH 6.8 and pH 1.2for increasing the pore size of it. The other end of tubing was also secured with thread and was allowed to rotate freely in the dissolution vessel of a USP type II dissolution test apparatus (Electro lab TDT-08L Plus, India) that contained 900 ml dialyzing medium (phosphate buffer 6.8/ pH1.2) maintained at 37±0.5˚C and stirred at 100 rpm. Placebo formulation (blank SMEDDS, without drug) was also tested simultaneously under identical conditions so as to check interference, if any. Aliquots were collected periodically and replaced with fresh dissolution medium and analyzed spectrophotometrically at 246 nm for atorvastatin content.

RESULTS AND DISCUSSION

Pseudo-ternary Phase Diagrams Study

The pseudo-ternary phase diagrams were mapped with the water titration method to identify the area of microemulsion regions at 37°C. The distilled water was used as diluting medium and added into the formulation. The proper ratio of one excipient to another in the SMEDDS formulation was analysed. Several formulations with different oil and Smix values (the ratio of surfactant to cosurfactant) were dispersed with water at 37°C. The pseudo-ternary phase diagrams of the formulation composed of Acrysol K 150, Gelucire 44/14 and PEG400 are shown in Figure 1. From figure, it was concluded that microemulsion region of ternary plot of ratio 1:1 Smix was greater than all other. The shadow area represents the o/w microemulsion existence region. The size of the micromulsion region in the diagrams was compared as, larger the size the greater the self-micro emulsification efficiency.

Drug Loading Capacity

Capacity of drug engulfment in microemulsion was observed by putting three different quantities and concludes that there is no precipitation of drug even after seven weeks and shows his maximum drug loading capacity by appealing the industrial use of SMEDDS. Preparation of liquid SMEDDS formulations In following experiment we undergo various trial batches so as to explore the perfect combination of excipients. After various trials prepared on the basis of solubility of drug, final batches were prepared through ternary phase diagram study shown in Table 2.

Preparation of Liquid SNEDDS Formulations

In following experiment we undergo various trial batches so as to explore the perfect combination of excipients. After various trials prepared on the basis of solubility of drug, final batches were prepared through ternary phase diagram study shown in Table 2.

Dilution Study

A right blend of emulsifier is necessary for the development of SMEDDS formulation; to form stable microemulsion. When 1 part SMEDDS of each solution was diluted with 10 parts of distilled water, HCl buffer 1.2 pH and phosphate buffer 6.8 pH (Table 3). It implies that the formulation was more stable because there was no precipitation.

SMEDDS Characterization

Thermodynamic Stability Studies

Various thermodynamic stability studies were performed by evaluating its temperature, centrifugation and dispersibility potential. A study reveals that there is no effect of any of the parameter on the transparency of microemulsion and not show any precipitation, phase separation, creaming or cracking given in Table 3.

Dispersibility Test

The efficiency of self-emulsification of formulation F1-F8 was shown in Table 4. All the formulations are of Grade A.

Table 4: Self Emulsification Time Values of Different Formulations

Formulation	Self emulsification time (sec.)	Grade
F1	28	A
F2	31	A
F3	35	A
F4	33	A
F5	37	A
F6	41	A
F7	39	A
F8	37	A

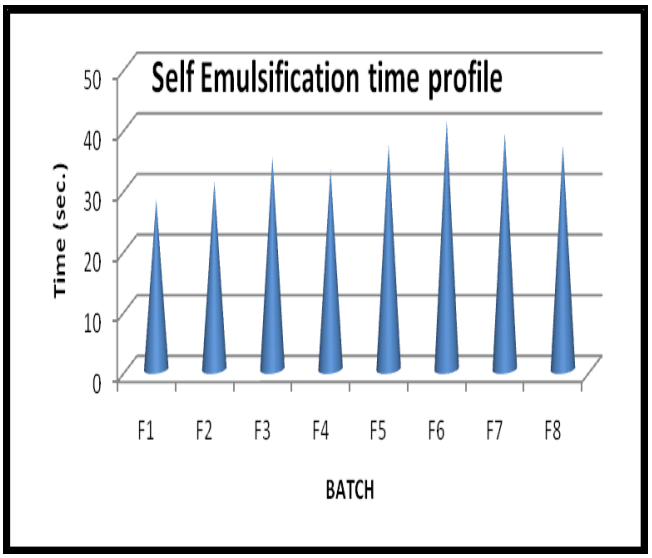

Figure 3: Self Emulsification Time of Various Formulations

Percentage Transmittance

Percentage transmittance of all batches in distilled water, HCl buffer pH 1.2 and Phosphate buffer pH 6.8 was above 97%, 98% and 95 %, respectively& for optimized batch it was above 99%. It indicates clear microemulsion was formed from the SMEDDS up to 100 times dilution with distilled water. It was shown in figure 4.

Table 5: Percent Transmittance Studies in Different Medium

Form^{n}	Percent Transmittance		
	In Distilled Water	In Buffer pH1.2	In Buffer pH6.8
F1	99.28±0.96	99.68±0.77	100.12±0.81
F2	100.15±0.62	99.62±0.32	99.26±0.55
F3	99.52±0.89	99.35±.0.64	98.74±1.54
F4	99.52±0.44	99.82±0.32	100.14±0.62
F5	99.46±2.16	99.23±0.68	99.57±0.45
F6	99.89±1.56	98.90±1.6	99.60±1.4
F7	97.5±1.20	99.62±0.48	95.82±1.89
F8	99.42±0.36	98.92±1.8	99.51±074

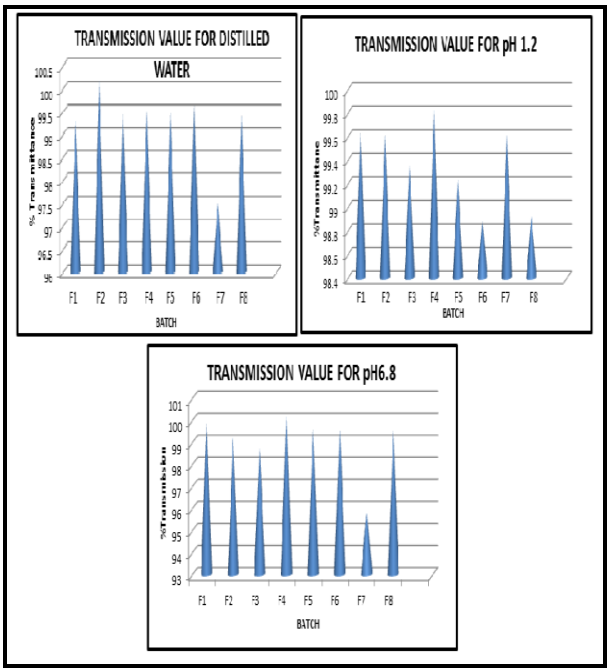

Figure4: Transmission Values of Formulations in Dist. Water, pH1.2, pH 6.8

Cloud Point Measurement Study

The cloud point is the temperature above which a clear formulation turns cloudy. At temperatures higher than the cloud point, an irreversible phase separation occurs due to dehydration of its ingredients, which may affect drug absorption. Hence, to avoid this phenomenon, the cloud point for SMEDDS should be above body temperature (37˚C). The cloud point for ATV SMEDDS was much higher (Above 65˚C) which indicates that it will form stable microemulsion at physiological temperature i.e. *in vivo*, without risk of phase separation shows in figure 5.

Table 6: Cloud Point Measurement Values of Different Formulations

Formulation	Cloud point (Temp. ˚C)
F1	74
F2	70
F3	77
F4	73
F5	78
F6	75
F7	69
F8	75

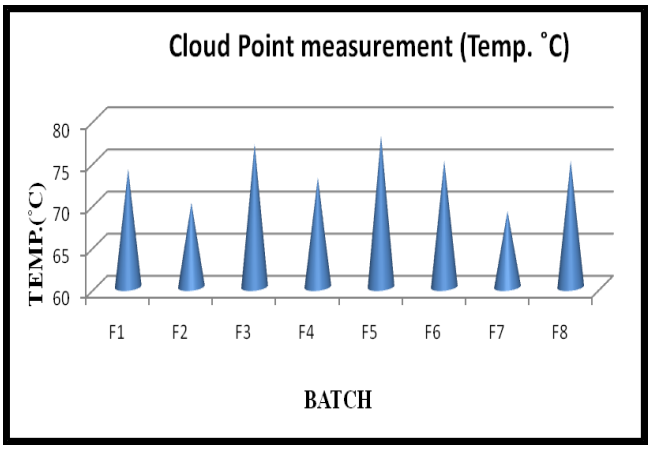

Figure 5: Cloud Point Measurement Study

Determination of Droplet Size/Distribution and Zeta Potential

Particle size after microemulsification was the most important property of SMEDDS. Mechanisms of particle size effect on drug absorption may include improved release and facilitated lymphatic transport. All batches showed mean globule size within range of 10.14nm-139.51 nm when diluted with distilled water. The time required for formation of microemulsion after dilution with distilled water was just 28 second. The resultant microemulsion were transparent in appearance and they didn't show any symptom of phase separation and drug precipitation even after 24 h. Droplet size of optimized formulation was given in figure 6.

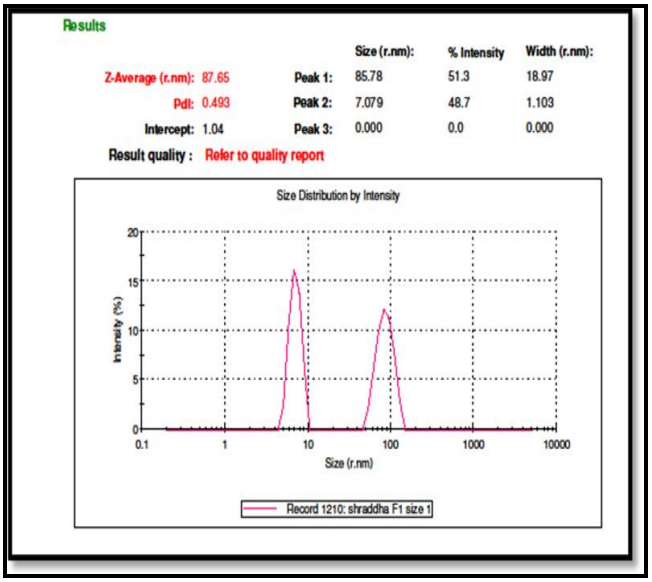

Figure 6: Droplet Size Analysis of Optimized Formulation F1

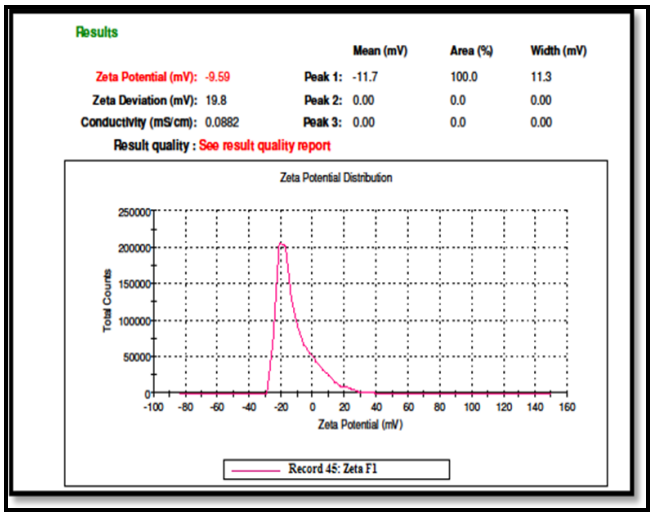

Figure 7: Zeta Potential of Optimized Formulation-F1

Zeta potential of the SMEDDS is helpful to recognize the charge of oil globules in the emulsion. The increase in electrostatic repulsive forces between the globules averts the coalescence of microemulsion. In contrast, decrease in electrostatic repulsive forces can cause phase separation. Several studies have reported that the zeta potential played an important role in the interactions with mucus of the gastrointestinal tract. The zeta potential of all the batches was negatives.& the zeta potential of the optimized formulation (F1) obtained by diluting with distilled water (100 times) was −9.59 mV and results are shown in Figure 7. The charge on an oil globule may be negative due to surfactants and/or cosurfactant present in the formulation.

Drug Diffusion Studies

Diffusion patterns of ATV from plain drug and various batches after reconstitution with phosphate buffer (pH 6.8) are depicted in Figure 8. The patterns disclose that release of ATV from various batches varied with change in pH. It was observed from optimized batch that more than 60% drug released within 5 min. and complete release was obtained within 20 min. in pH 6.8, while 90 % drug was released in 25 min. in pH1.2 buffer. This difference may be due to the difference in solubility of ATV at different pH. These results were found to be more sliding with plain drug having only release 30% in 60 min. Thus, we can say that the formulated SMEDDS having much potential than the plain drug and shows uniform *in vitro* release throughout the GIT irrespective of pH variations.

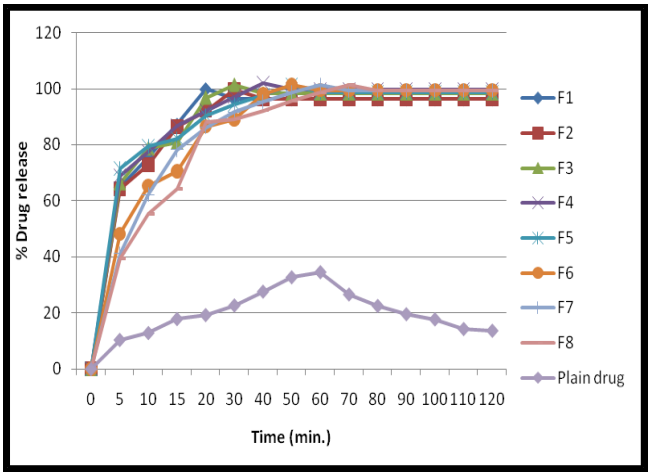

Figure 8: *In Vitro* Drug Diffusion of Formulation F1-F8 with Plain Drug

CONCLUSION

The present research work could be summarized as successful development of SMEDDS of Antihyperlipidemic ATV using Acrysol K150, Gelucire 44/14, PEG 400 which gives stable and transparent microemulsion. Based on higher solubility, ultimate micro-emulsifying zone, lesser globule size with mini. polydispersity index, acceptable globule charge, higher transmittance, higher cloud point and superior drug release illustrate the potential use of Atorvastatin SMEDDS orally. Formulation can also be given in liquid solution or in capsule dosage form deemed to be the efficacious and patient compliant delivery system. Studies also showed how microemulsion formulation can be supportive for the delivery of hydrophobic compounds.

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