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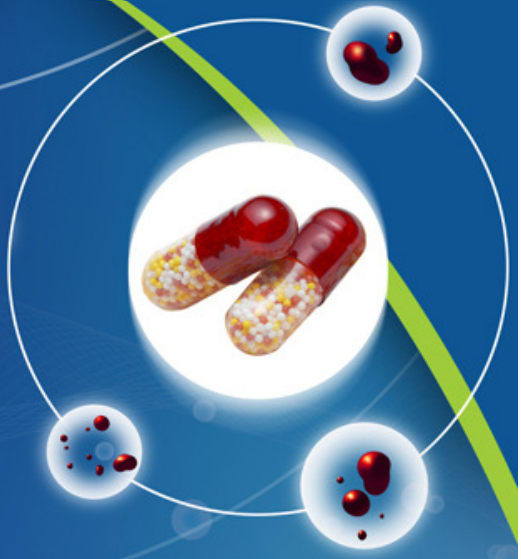

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Research Article

FORMULATION AND EVALUATION OF SUPERSATURABLE SELF NANO-EMULSIFYING DRUG DELIVERY SYSTEM OF POORLY WATER SOLUBLE ATORVASTATIN CALCIUM.

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ABSTRACT:

The SSNEDDS approach is to generate a protracted supersaturated solution of the drug when the formulation is released from an appropriate dosage form into an aqueous medium. Surpersaturation is intended to increase the thermodynamic activity to the drug beyond its solubility limit and, therefore, to result in an increased driving force for transit into and across the biological barrier. The drug will be solubilized in the GI tract in oil droplets, the large amount and small size of which lead to a considerable increase of surface area from where drug dissolution can take place. SNEDDS can enhance drug absorption by a number of ancillary mechanisms, including reduction of gastric motility and alteration of the physical and biochemical barrier function of the gastro-intestinal mucosa. Supersaturable SEDDS formulations differ from the conventional SEDDS formulations as they contain a reduced amount of surfactant and a polymeric precipitation inhibitor.

KEYWORDS: *Supersaturated Self Nano-emulsifying Drug Delivery System (SSNEDDS), Atorvastatin Calcium. PVP.*

INTRODUCTION:

More than 40% drugs are poorly water soluble, hence different types of method are applied for increasing their solubility as well as bioavailability of drugs. The properties of new chemical entities shifted towards higher molecular weight and increasing lipophilicity, resulting in decreased aqueous solubility. An example of such a recently discovered compound suffering from poor bioavailability is the highly potent drugs.[1,2]

SNEDDS is defined as isotropic mixtures of oil and surfactant and co-surfactants that form o/w nanoemulsion upon mild agitation followed by dilution in aqueous media, such as GI tract.

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High amount of surfactant provided to avoid precipitation of drug following by dilution with water in the GI tract but high amount of surfactant causes irritation to GIT.[3,4,5,6]

The SSNEDDS formulations contain a reduced level of surfactant and a polymeric precipitation inhibitor to yield and stabilize a drug in a temporarily supersaturated state. Supersaturation is intended to increase the thermodynamic activity to the drug beyond its solubility limit and, therefore, to result in an increased driving force for transit into and across the biological barrier. The supersaturable SNEDDS was designed, using a small quantity of PVP (or other polymers) in the formulation to prevent precipitation of the drug by generating and maintaining a supersaturated state *in vitro*.[7,8]

Atorvastatin Calcium is a member of the drug class known as statins and comes under BCS class II drugs category. It is used for lowering cholesterol. Atorvastatin calcium is a competitive inhibitor of hydroxyl methyl glutaryl-coenzyme A (HMG-CoA) reductase, the rate-determining enzyme in cholesterol biosynthesis via the mevalonate pathway. HMG-CoA reductase catalyzes the conversion of HMG-CoA to mevalonate.[9]

MATERIAL AND METHOD:

Material:

Atorvastatin Calcium (Wockhardt Pharm., Aurangabad) Oleic acid (Qualigens Fine Chemicals Ltd.) Tween 20 Transcutol-P (GattefossePharma Ltd.), PVP K30, Beta-cyclodextrin (S.D. fine chem Ltd. Mumbai).

Method:

Solubility studies:

Unknown amount of selected vehicles was added to each cap vial containing an excess of drug. After sealing, the mixture was heated at 40°C in a water bath to facilitate the solubilization. Mixing of the systems was performed using a vortex mixer. Formed suspensions were then shaken with a shaker at 25°C for 48 hours. After reaching equilibrium, each vial was centrifuged at 3000 rpm for 5 minutes, and excess insoluble ATOR was discarded by filtration using a membrane filter (0.45µm Whatman, India). The concentration of drug was then quantified by UV-Spectrophotometer.

Solubility of Atorvastatin calcium in various oils, surfactants and co-surfactants are shown in Figure 1, 2 and 3 respectively.

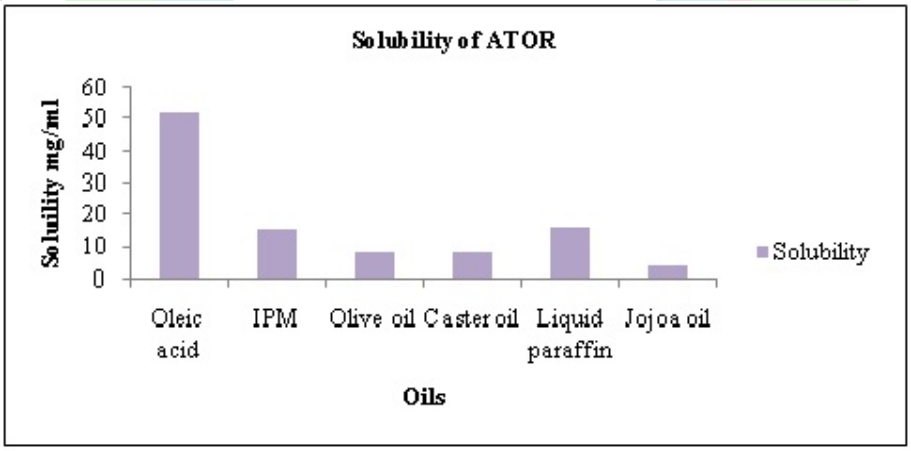

Figure 1: Solubility of Atorvastatin calcium in Oils.

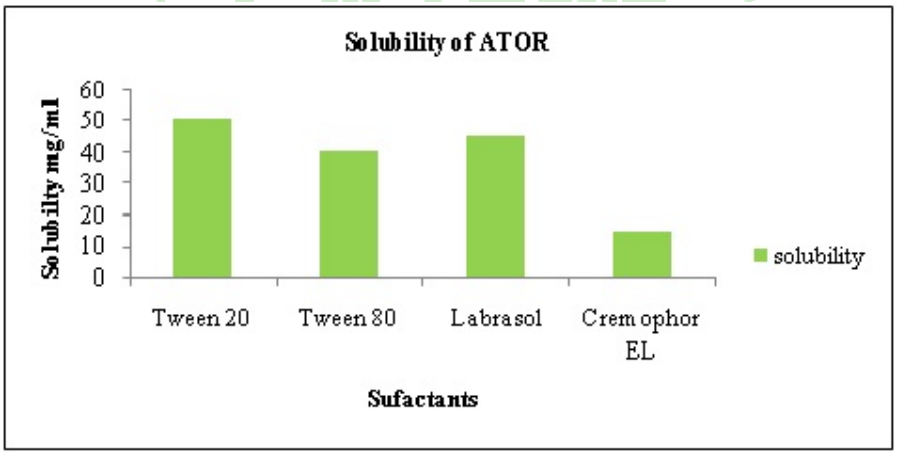

Figure 2: Solubility of Atorvastatin calcium in Surfactants.

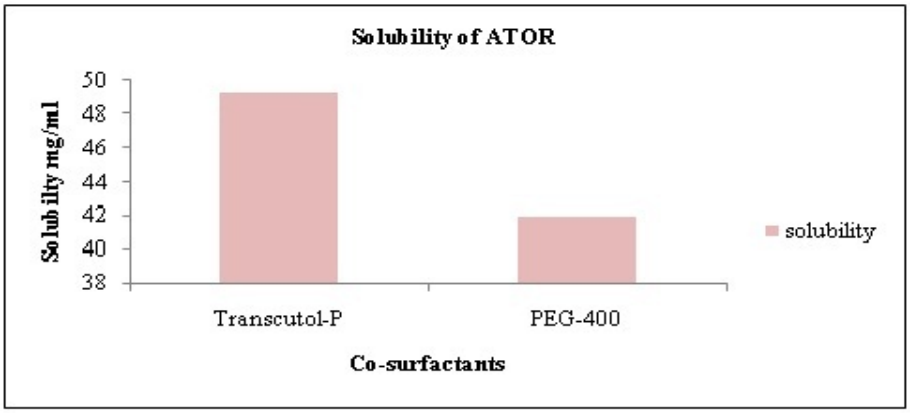

Figure 3: Solubility of Atorvastatin calcium in Co-surfactants.

Study on phase behavior using Water Titration Method:

Procedure:

For each phase diagram, oil and specific Smix ratio was mixed thoroughly in different volume ratios from 1:9 to 9:1 in different small glass test tubes. Sixteen different combinations of oil and 9each Smix, 1:9, 1:8, 1:7, 1:6, 1:5, 2:8 (1:4), 1:3.5, 1:3, 3:7 (1:2.3), 1:2, 4:6 (1:1.5), 5:5 (1:1), 6:4 (1:0.7), 7:3 (1:0.43), 8:2(1:0.25), 9:1 (1:0.1) were made so that maximum ratios were covered for the study to delineate the boundaries of phases precisely formed in the phase diagrams. For the determination of existence zone of nanoemulsion, pseudo ternary phase diagrams were constructed using water titration method. To construct pseudoternary phase diagrams, the oil phase (oleic acid: Tween 20 1:1) was mixed with different ratio of surfactant and co-surfactant (Tween 20 and Transcutpl-P respectively) and mixture was titrated with distilled water until it turned turbid. The ratio of surfactant and co surfactant (Tween 20 and Transcutol-P) were used for the titration are, 1:0,1:1,1:2,1:3,2:1,3:1 and 4:1 respectively.

Preparation of Liquid Super Saturated SNEDDS:

On the basis of the "Solubility studies" section, the oil (oleic acid), surfactant (Tween20), and co-surfactant (Transcutol-P) were selected due to their greater solubility enhancement effect on Atorvastatin Calcium. Various formulations were tried as shown in Table 1. The formulations were prepared by dissolving Atorvastatin Calcium in the mixture of oil, surfactant, co-surfactant and polymer and were mixed by gentle stirring on magnetic stirrer for 15 mins, until a transparent preparation was obtained. All the mixtures were stored at room temperature for further use.

Table 1: Formulation of Atorvastatin Calcium Liquid Ssnedds.

Formulation	Oleic acid (%)	Tween 20 (%)	Transcutol-P (%)	Polyethylene glycol (%)	Atorvastatin Calcium (mg)
F1	20	40	40	-	20
F2	10	45	45	-	20
F3	10	60	30	-	20
F4	20	60	20	-	20
F5	20	40	-	40	20

F6	10	45	-	45	20
F7	10	60	-	30	20
F8	20	60	-	20	20

Preparation of solid SNEDDS:

β-cyclodextrin (2gm) was dissolved in 100 ml water by magnetic stirring. The solution was filtered by whatman filter paper to remove undissolved particles. The liquid SNEDDS was then added to β-cyclodextrin with constant stirring for 20 min. The emulsion was dried with lab scale spray dryer (Labultima U-222).

RESULTS AND DISCUSSION:

Evaluation of SNEDDS:

Dispersibility Test and Determination of Self Emulsification Time:

It was carried by using a standard USP dissolution apparatus 2, formulation was added to 500 ml of water at 37 ± 0.5 ºC and the paddle was rotated at 50 rpm. On titration with water the SNEDDS formulation forms a mixture which was of different type given in Table 2 which was depending upon the *in vitro* performance of formulation can be assessed.

Table 2: Type of Formulation depending upon visual observation type of Formulation.

Sr. No.	Dispersibility and Appearance	Grade	Time to SE (min)
FI	Dull	C	Within2
F2	Clear and transparent	A	Within 1
F3	Clear	A	Within 1
F4	Translucent	B	Within 1
F5	Dull	C	Within 2
F6	Clear and transparent	A	Within 1
F7	Clear	A	Within 1
F8	Translucent	B	Within 1

Viscosity:

The viscosities of the prepared nanoemulsion formulations were determined as such without dilution by Brookfield viscometer using spindle # CPE61 at 25±0.5°C. The parameters, which were set after optimizing the procedure, were listed in the Table 3.

Table 3: Viscosity, % Transmittance and % Drug Content of all the Formulations.

Formulation	Viscosity (cp)	% Transmittance	% Drug Content
F1	24.87±0.54	97.12±0.021	95.32±0.061
F2	32.65±0.27	99.32±0.016	98.89±0.046
F3	21.84±0.64	98.58±0.009	96.64±0.029
F4	45.95±0.45	98.73±0.011	94.96±0.015
F5	39.63±0.23	97.56±0.053	95.36±0.033
F6	29.04±0.54	99.12±0.054	97.74±0.034
F7	19.74±0.65	98.64±0.043	95.84±0.032
F8	27.99±0.43	96.32±0.011	96.42±0.042

Transmittance Test:

1ml of formulation was diluted 100 and 1000 fold with water (ml), and % transmittance was determined using UV spectrophotometer at 630 nm. (Table 3)

% Drug Content:

The absolute drug content of nanoemulsion containing Atorvastatin Calcium was determined by UV-Spectrophotometer at 246nm shown in Table 3.

1ml of liquid SNEDDS was dissolved in

100 ml Methanol

Stirring

↓

The solution was filtered using

0.45 µm membrane

↓

Filter

The drug content was determined by UV method.

Thermodynamic Stability Studies:

Heating cooling cycle: **-** Six cycles of cooling and heating between refrigerator temperature (4°C) and elevated temperature (45°C) with exposure at each temperature for not less than 48 hours are carried. That formulation, which was stable, was then subjected to centrifugation test.

Centrifugation: **-** Formulations which pass the heating cooling cycle was centrifuged at 3500 rpm for 30 min. That formulation that doesn't show any phase separation was taken for the freeze thaw stress test.

Freeze thaw stress cycle:- Three freeze thaw cycles between -21° C & 25° C with storage at each temperature for not less than 48 hours. Those formulations which pass this test show good stability with no phase separation, cracking or creaming.
The physical stability of a formulation was very important for its performance as it can be adversely affected by precipitation of the drug in excipient matrix. Thermal stability showed in Table 4.

Table 4: Thermodynamic Stability of all the Formulations.

Formulation	Heating cooling cycles	Centrifugation	Freeze thaw stress cycle
F1	×	×	×
F2	√	√	√
F3	√	√	√
F4	√	√	×
F5	×	×	×
F6	√	√	√
F7	√	√	√
F8	√	√	×

Droplet Size Analysis and Particle Size Measurements (Globule Size):

It is a precise method for evaluation of stability. All measurements were carried out at scattering angle of 90° and 25°C temperatures. Prior to measurement, nanoemulsion was diluted in two-steps with pure water then it was filtered through a 0.22µm filter just before it was added to cuvette. Globule size and polydispersity of nanoemulsion shown in Table 5.

Table 5: Globule Size and Polydispersity of all the Formulations.

Formulation	Mean Globule size(nm)	Polydispersity	Mean Zeta Potential (mV)
F1	70	0.215	-2.94
F2	21.6	0.315	-17.78
F3	41.5	0.206	-3.55
F4	56.4	0.204	-6.95
F5	65.3	0.308	-1.45
F6	43.5	0.285	-16.84
F7	73.6	0.335	-4.46
F8	414.	0.247	-3.89

Zeta Potential Measurement:

Samples were placed in clear disposable zeta cells and were monitor at 25°C at a scattering angle of 90°C results were recorded. Zeta potential of spray dried powder was -17.78 for F2 formulation which is shown in Figure 4.

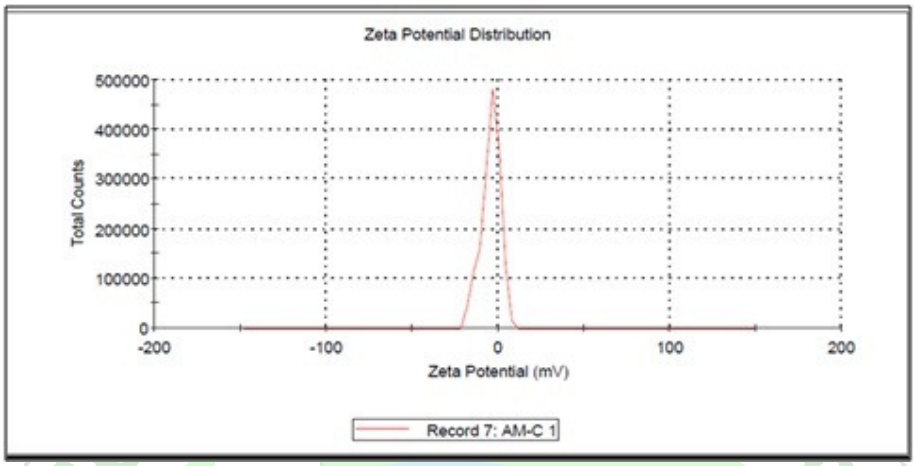

Figure 4: Zeta Potential of F2 formulation.

Differential Scanning Calorimetry:

Differential scanning calorimetry for SNEDDS can be determined using DSC. Liquid sample and Solid sample was placed in the aluminum pan.

The DSC thermogram of pure Atorvastatin Calcium showed the endothermic peak at 159.27°C indicated the melting point. (Figure 5) The DSC thermogram of F2 formulation of Spray dried powder and liquid (F2) shown peak at 165.22°C. (Figure 6) the endothermic peak at 159.02°C (Figure 7) respectively, thus the DSC thermogram of drug was found to be agreement to the specification.

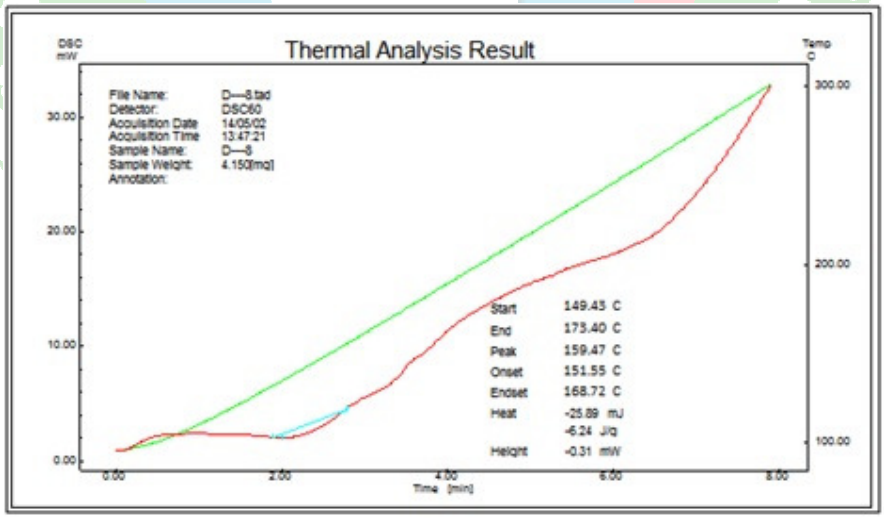

Figure 5: DSC Thermogram of Atorvastatin Calcium API.

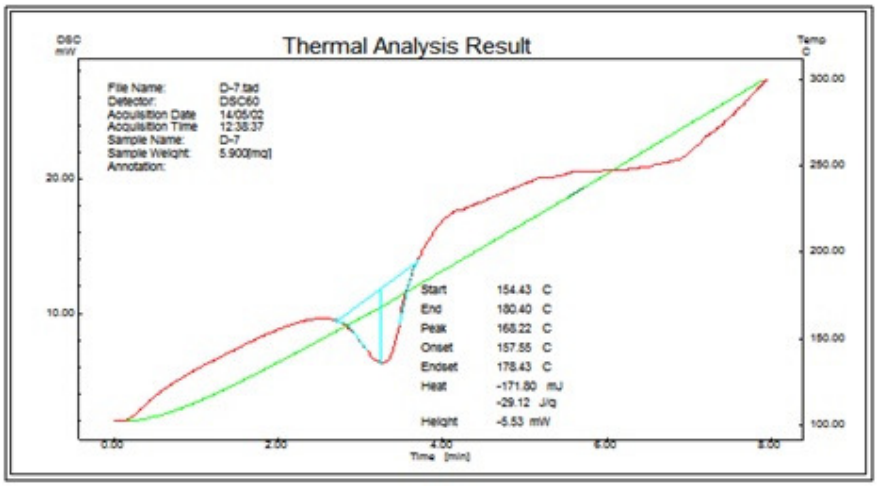

Figure 6: DSC Thermogram of F2 formulation (Solid).

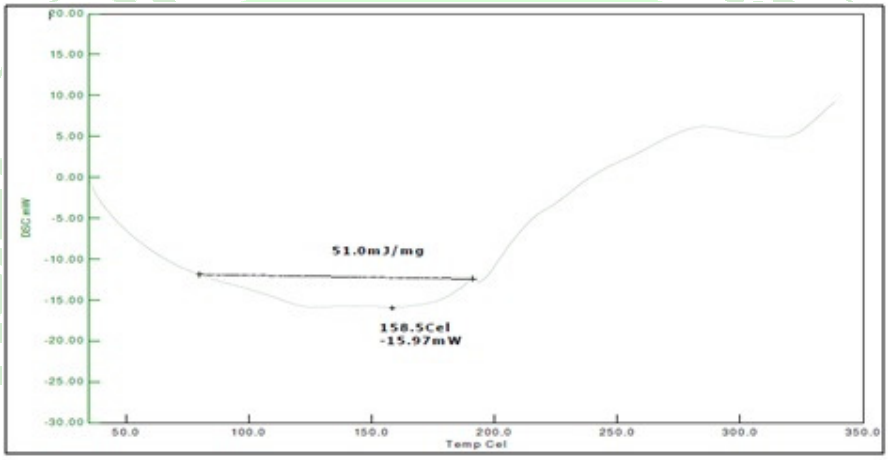

Figure 7: DSC Thermogram of F2 formulation (Liquid).

Scanning Electron Microscopy:

The formulation F2 was analyzed by Scanning Electron Microscopy for studying particle shape and surface structure for both liquid and solid also. The shapes of formulation are shown in Figure 8 and 9.

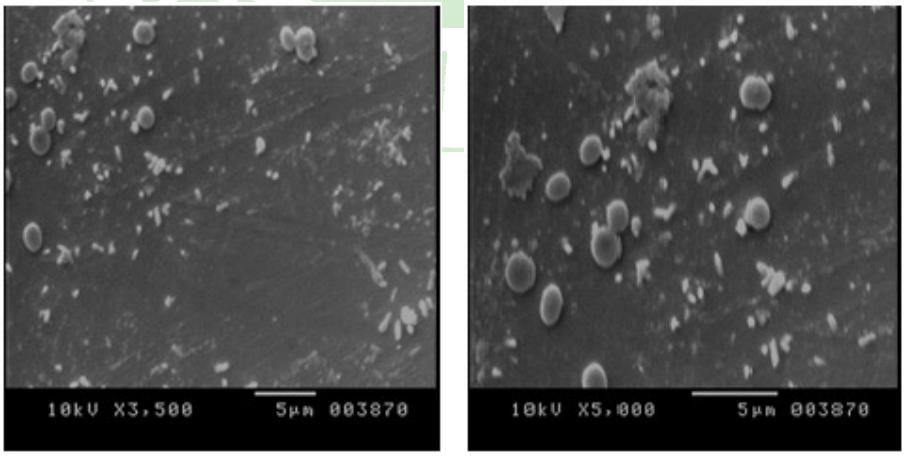

Figure 8: SEM of Formulation of F2 (Liquid).

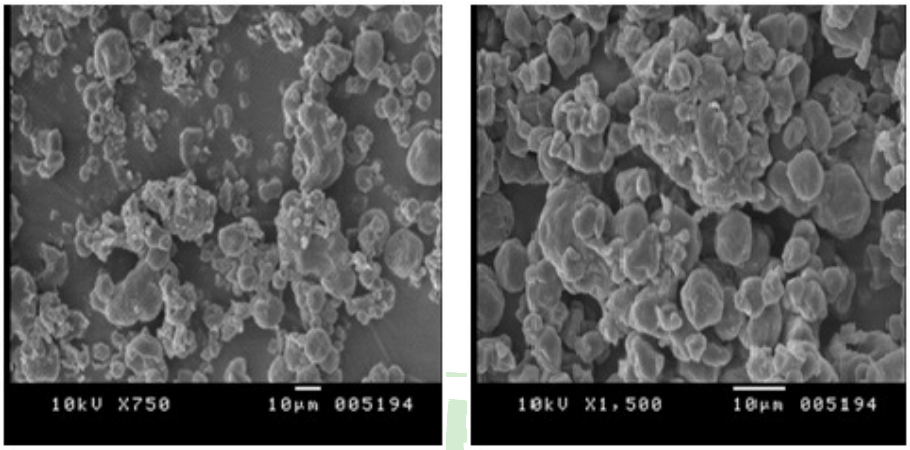

Figure 9: SEM of Formulation of F2 (Solid).

X-Ray Diffraction:

X-rays are electromagnetic radiations having a wavelength of about 1°A, which is approximately the size of an atom. It is used for analysis of crystalline solids at an atomic level. X-ray diffraction of spectra of Atorvastatin calcium has sharp at different diffraction angle, which showed typical crystalline pattern. Atorvastatin calcium spray dried powder shows peaks of low intensity indicating that some amount of drug converted in to amorphous form.(Figure 10)

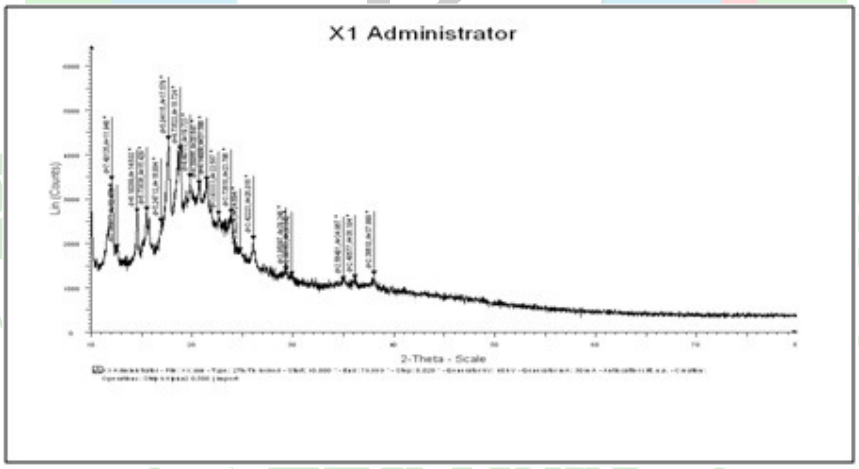

Figure 10: X-ray Diffraction of F2 Formulation.

In-Vitro Dissolution Test:

Quantitative *in-vitro* release test was performed using USP basket type dissolution test apparatus (ELECTROLABS, USP standard) at 50 rpm and 37 ± 0.5°C in 900 ml phosphate buffer at pH 6.8. SSNEDDS (equivalent to 20 mg in capsule Shell). Percentage cumulative drug released at different time intervals was calculated and graph plotted % drug release versus time. The total percentage release was much higher for SSNEDDS than pure Atorvastatin calcium *In-vitro* dissolution study of formulation (F1 to F8) showed % drug release from 87.609% to 96.971% (Figure 11). Whereas pure Atorvastatin calcium showed 63.797% of drug release in 60 mins shown in Figure 12.

Table 6: *In -Vitro* Drug Release of all Formulations compared with ATOR API

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Sr. No.	Time in mins	% Drug Release								
		F1	F2	F3	F4	F5	F6	F7	F8	API
1.	0	00.00	00.00	00.00	00.00	00.00	00.00	00.00	00.00	00.00
2.	5	6.388	10.472	3.049	3.229	5.111	5.492	5.738	3.170	1.049
3.	10	9.520	14.782	4.151	5.278	9.051	20.535	7.867	12.538	6.287
4.	15	11.815	17.092	11.755	6.425	12.903	23.502	16.367	17.824	11.767
5.	20	15.138	23.002	17.212	12.835	20.318	29.079	34.032	21.502	15.089
6.	25	25.779	32.093	25.732	22.331	28.292	32.354	38.373	24.822	25.732
7.	30	32.926	38.213	35.014	33.589	41.729	39.406	45.800	32.871	30.825
8.	35	39.940	45.609	39.904	40.671	46.256	46.353	47.856	39.774	33.475
9.	40	47.099	54.748	45..98	47.671	54.093	52.657	50.459	48.810	39.531
10.	45	57.286	62.319	56.177	53.631	56.343	62.388	61.467	72.357	41.188
11.	50	66.034	72.725	65.987	64.417	70.596	75.171	72.928	77.722	47.124
12.	55	72.800	85.972	76.752	74.356	79.462	82.309	83.293	82.993	56.242
13.	60	87.609	96.971	90.291	85.096	92.445	93.022	91.317	93.342	63.797

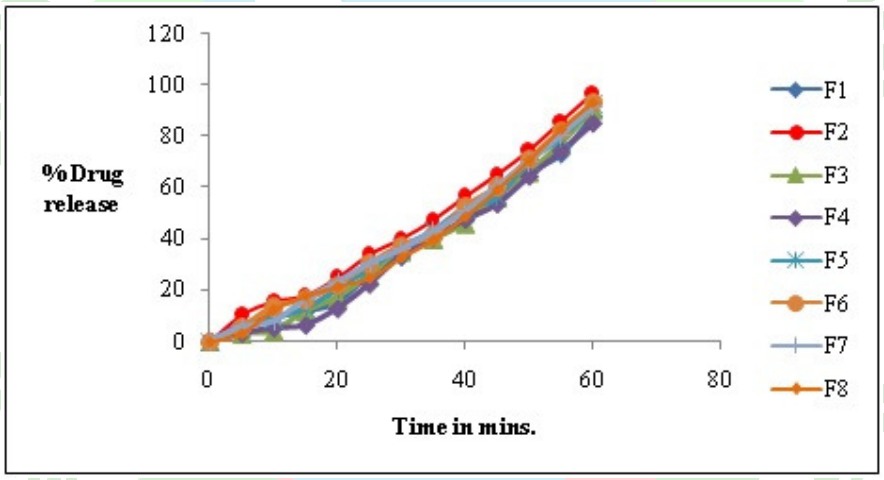

Figure 11: Comparative In-vitro Drug Release of all Formulation.

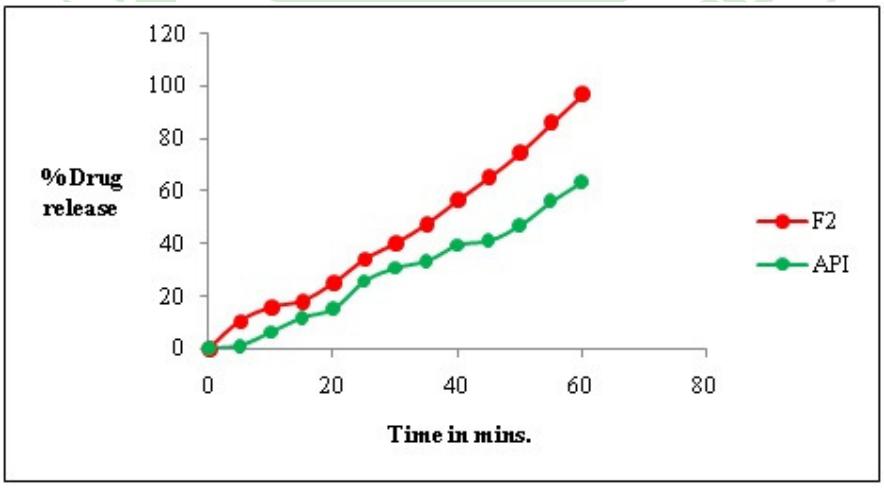

Figure 12: Comparative *In-vitro* Drug Release of F2 Formulation with ATOR API.

Release kinetics and Mechanism:

To know the release mechanism and kinetics of Atorvastatin calcium optimized formulations (F2) was attempted to fit into mathematical models and n, r2 values for zero order, first order, matrix Korsmeyer-Peppas and Hixon- Crowel models were represented in Table 7.

Zero-Order Release kinetics:

For zero-order release kinetics, the dissolution of a drug molecule is only a function of time. This model holds true only in the case of very slow drug release. (Figure 13) Zero-Order release is therefore modeled as follows:

$$M0-Mt = k0t$$

Where,

$M0$ is the initial concentration of drug present in the drug molecule,

Mt is the concentration of drug in the drug molecule at time t,

$k0$ is the Zero-Order release constant with units of concentration per time.

Table 7: *In-Vitro* Drug Release Kinetics of F2 Formulation.

Models	R^2 value	K value
Zero order	0.9976	9.9210
1st order	0.8426	-0.2470
Matrix	0.9374	25.8345
Korsmeyer- Peppas	0.9920	13.4878
Hixon- Crowel	0.9368	-0.0559

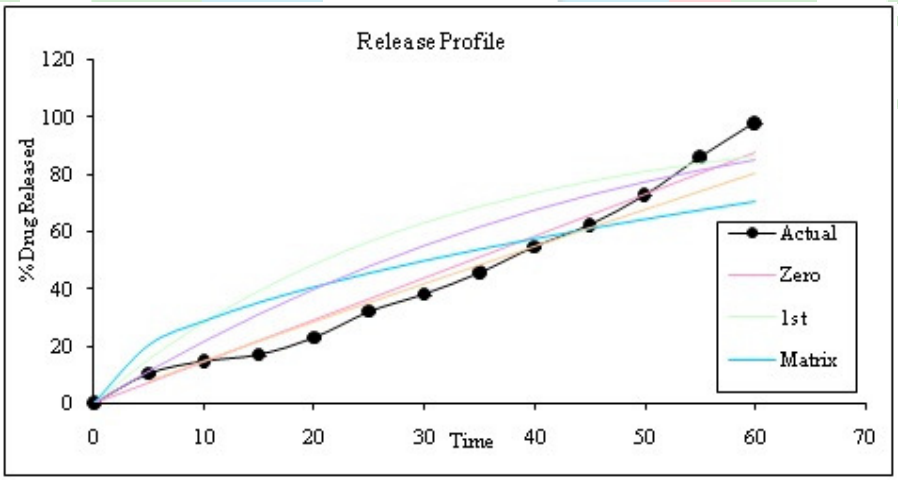

Figure 13: *In-vitro* Drug Release (Zero-order) Kinetics of F2 formulation.

CONCLUSION:

Differential Scanning calorimetric data indicated that there was no probable of interaction between drugs and excipients. Zeta potential of Optimized formulation (F2) was found to be -17.78 mV which indicates high negative surface charge on

particle which in turn indicates higher stability. Liquid SNEDDS convert in to solid SNEDDS by spray drying method successfully. The total percentage release was much higher for SSNEDDS (F2) than pure Atorvastatin calcium as compare to API. The dissolution study indicates that it is promising system for the enhancement of solubility and bioavailability of drugs.

The mean particle size of Liquid nanoemulsion ranged from was 21.6nm to 73.6nm. SEM photographs of the nanoemulsion revealed that the nanoemulsion showed spherical shape. The F2 formulation shows 98.89% drug release from the nanoemulsion formulation in1hr. The drug release profiles of the Atorvastatin Calcium nanoemulsion formulation showed best fit with Zero Order kinetics model.

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