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Formulation, Development, Characterization and in Vitro Study of Rosuvastatin Calcium Microemulsion

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ABSTRACT

The purpose of conducting this study was to prepare a microemulsion formulation of Rosuvastatin calcium (RC) for transdermal drug delivery. Oil in water microemulsion was formulated using Isopropyl myristate, Tween 20, and Propylene Glycol as oil, surfactant, and co-surfactant, respectively. The ideal proportion of surfactant: co-surfactant (Smix) was chosen by constructing pseudoternary diagrams. The microemulsion formulations, which proved to be stable after thermodynamic stability testing, were further evaluated for physical characteristics. Selected formulations were evaluated for droplet size, zeta potential, viscosity, and % drug content. Results suggested that optimized microemulsion formulation was thermodynamically stable and clear, droplet size and zeta potential was determined. In-vitro dissolution study for optimized microemulsion was performed and cumulative % drug release was determined.

KEYWORDS: *Microemulsion, Transdermal drug delivery, Rosuvastatin calcium*

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INTRODUCTION[1, 10]

Hyperlipidemia is a serious illness, including abnormal blood levels of various lipids, specifically cholesterol, triglycerides, phospholipids, and/or plasma lipoproteins. It is a major contributor to atherosclerosis and a number of other serious cardiovascular diseases (CVDs). The prevalence of high lipid concentrations in hyperlipidemic patients is of great concern to the medical community because CVD-related deaths are on the rise having grown more recent. The effects could be so severe that in the upcoming year, CVDs will become one of the leading causes of death worldwide. Patients who are at a higher risk for CVDs are treated with lipid-lowering medications or undergo lifestyle changes, such as dietary adjustments, increased physical activity, etc., to control lipid levels in the body. Low-density lipoprotein levels can be decreased and CVDs can be avoided in those who are at risk due to statins. In individuals with hyperlipidemia, rosuvastatin, a highly effective statin medicine, is used to lower blood levels of cholesterol, triglycerides, etc. This can aid in slowing the development of atherosclerosis, which is recognized to be a strong risk factor for CVDs.

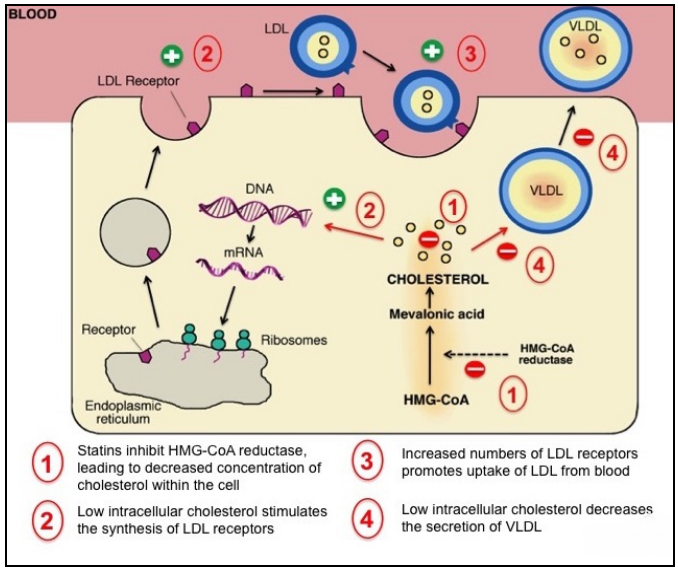

Figure 1: Mechanism of action of Rosuvastatin Calcium

As of right now, the only oral preparations of RC on the market are tablets and capsules. The primary issue with its oral forms is that it is a BCS class II medication with low solubility in water. Consequently, a new formulation of to overcome its solubility and bioavailability, RC is required issues. A microemulsion is a clear, thermodynamically stable colloidal dispersion made spontaneously from a mixture of oil, water, and surfactants. Lipid-based microemulsions can be used to enhance the bioavailability of RC if administered through transdermal drug delivery system. Many reports in the literature suggested that the use of microemulsions can enhance bioavailability of loaded drugs by preventing enzymatic degradation and improving membrane permeability. The investigation was focused on preparing and optimizing oral microemulsion of RC by using physicochemical parameters such as percent transmittance, droplet size, zeta potential, viscosity, etc. The selected formulation was then studied for in-vitro release of RC from the microemulsions.

MATERIALS AND METHODS

Materials

RC was a procured from Aarti Distributors, Mumbai, India. Isopropyl myristate (), castor oil (), Tween 20 (), Tween 80 (), Tween 40 (), Span 20 (), Span 40 () and propylene glycol () were generous gift from Croda India Company Pvt. Ltd, Maharashtra, India. Olive Oil, Peppermint oil, Soybean Oil, Cotton Seed Oil, Castor Oil, Dill Oil, Almond Oil, Anise seed Oil, Jojoba Oil, Coconut Oil, PEG-400, Ethanol, Glycerol and Phosphate Buffer pH 7.4 were procured from Research lab Fine Chemicals Industry, Mumbai, India. The chemicals used were of analytical reagent grade.

UV Spectroscopy [18]

Preparation of standard solution and determination of λ max of Rosuvastatin calcium

10 mg of Rosuvastatin Calcium was accurately & dissolved completely in 100 ml phosphate buffer pH 7.4 to get 1000 µg/ml (1 mg/ml) stock solutions. From it, pipette out 10 ml (1mg /ml) was further diluted with phosphate buffer pH 7.4 to obtain solution of 100 µg /ml. The aliquots were scanned for max from 200 to 400 nm on UV spectrophotometer.

The λ max was determined in phosphate buffer pH 7.4 solution.

Calibration curve of Rosuvastatin calcium in phosphate buffer pH 7.4 and methanol

Calibration curve of drug Rosuvastatin calcium was prepared in phosphate buffer pH 7.4 and methanol. Accurately weighed 100 mg of drug was dissolved in 100 mL of respective media (1000 µg/mL). From this solution 10 mL solution was pipette out in 100 mL of volumetric flask and volume was made (100µg/mL). From the above stock solution, aliquots of 0.2ml 0.4ml, 0.6ml, 0.8ml, 1ml were transferred to series of 10 mL of volumetric flask and volume was made with different media to get serial dilutions containing 2-10 µg/mL of drug substance. The absorbance values recorded using Shimadzu, UV-visible 1800 spectrophotometer at 242 nm.

Screening of excipients by using solubility studies [13]

The oil, surfactant, and co-surfactant were selected on the basis of solubility of RC in various excipients. Solubility was analyzed by adding a surplus amount of drug in about 2 mL of selected excipients by thoroughly blending them in vials using a vortex mixer. The vials were further ultra-sonicated for 72 hours to equilibrate the samples and then centrifuged at 5,000 rpm for 10 minutes. The supernatant fluid obtained was filtered and analyzed for the concentration of RC in samples using an ultraviolet spectrophotometer at 242 nm.

Drug-excipient compatibility study [21]

The surfactants used for the preparation of microemulsion must be evaluated beforehand for their interactions with the drug. For this purpose, a known amount of drug was mixed with various excipients who gained maximum solubility in solubility studies. Isopropyl myristate, tween 20 and propylene glycol were mixed with KBr (1:1) and prepared pellets were analyzed for the determination of drug- excipient compatibility in Infrared Spectrophotometer Shimadzu FTIR-8400S.

Development of pseudoternary phase diagrams [15, 16]

Pseudoternary diagrams were prepared to characterize the microemulsion region and to find out the optimum combination of components (oil, surfactant, and co-surfactant) used for the preparation. The water titration method was used, wherein fixed proportions of surfactant: co-surfactant (Smix), i.e., 1:1, 2:1, 3:1 and 1:2 w/w was taken. Smix and oil were mixed in a ratio of 1:9, 1:8, 1:7, 1:6, 1:5, 1:4, 1:3, 1:2, 1:1, 2:1, 3:1, 4;1, 5:1, 6:1, 7:1, 8:1 and 9:1 and water are added drop wise to each oil-Smix mixture under continuous moderate stirring. The mixtures were assessed visually when it changes from clear to opaque, indicating the traversing from microemulsion to coarse emulsion zone. The plotting pseudoternary diagrams were done using MS Excel.

Thermodynamic stability [26]

A. **Heating cooling cycle**: Six cycles between refrigerator temperature 4˚C and 45˚C with storage at each temperature of not less than 48h

was studied. Those formulations, which were stable at these temperatures, were subjected to centrifugation test.

B. Centrifugation: Passed formulations were centrifuged at 3500 rpm for 30min. Those formulations that did not show any phase separation were taken for the freeze thaw stress test.

C. Freeze thaw cycle: Three freeze thaw cycles between 4˚C and +25 ˚C with storage at each temperature for not less than 48h was done for the formulations. Those formulations, which passed these thermodynamic stress tests, were further taken for the dispersibility test for assessing the efficiency of self-emulsification. The formulations were observed visually for any phase separation or color change.

CHARACTERIZATION OF MICROEMULSIONS

Appearance

By visually inspecting the formulation under light alternately against white and black backgrounds for the existence of turbidity, the appearance was determined. When compared to pure water, the transparency of microemulsions was measured in terms of percent transmittance. Using a UV-visible spectrophotometer, the percent transmittance of a known amount of formulation was multiplied by 100 and distilled water.

PHYSICAL CHARACTERISTICS OF MICROEMULSION

Percentage Drug Content of Formulation [16, 17]

The percentage drug content of the formulation was analyzed by dissolving 1 ml of the formulation in 10 ml phosphate buffer pH 7.4. After suitable dilutions with methanol, absorbance was determined using the UV spectrophotometer (UV 1800, Shimadzu, Japan) keeping blank microemulsion as control at wavelength 242 nm.

Particle size analysis & Zeta-potential analysis [21]

For the determination of droplet size and zeta potential the prepared formulations were suitably diluted with distilled water. To ensure complete dispersion of the formulation, the samples were inverted twice. Following complete dispersion, the microemulsions were subjected to HORIBA SZ-100 for the droplet size determination. The principle involved is due to Brownian motion of droplets as a function of time which is determined due to fluctuation in light scattering, and it determines by photon correlation spectroscopy.

In-vitro drug release study [24]

Franz diffusion cells with a cellulose membrane were utilized to determine the Release rate of Rosuvastatin calcium from different microemulsion formulations. The cellulose (molecular weight G12 000) membrane was first hydrated in the distilled water solution at 25 ^{0}C for 24 hours. The membrane was then clamped between the donor and receptor compartments of the cells Diffusion cell was filled with 25 ml of phosphate buffer (pH = 7.4) and methanol (1:2). The receptor fluid was constantly stirred by externally driven magnetic bars at 600 rpm throughout the experiment. Rosuvastatin calcium microemulsion (5 g) was accurately weighted and placed in donor compartment. At 0.5, 1, 2, 3, 4, 5, 6, 7, 8 and 24 hr time intervals, 2 ml sample was removed from receptor for spectrophotometric determination and replaced immediately with an equal volume of fresh receptor solution. Samples were analyzed by UV visible spectrophotometer at 242 nm. The results were plotted as released drug percent versus time.

RESULTS AND DISCUSSION

UV Spectroscopy

The UV spectrum of Rosuvastatin Calcium was obtained in methanol as a solvent which shows absorbance maxima at wavelength 242 nm.

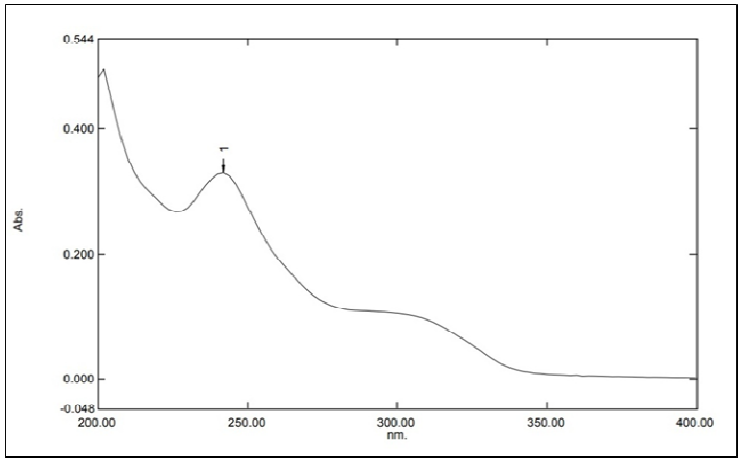

Figure 2: Absorbance maxima at wavelength 242 nm of RC

Calibration curve by UV Spectroscopy

The calibration curve of the Rosuvastatin Calcium was prepared in phosphate buffer pH 7.4 and methanol. 10.1 shows the absorbance at λ max 242 nm for different concentrations of Rosuvastatin Calcium and Figure 10.2 and Figure 10.3 shows the calibration curve. The regression coefficient was found to be 0.992 with slope value 0.072 and the Y intercept value 0.009 in phosphate buffer pH 7.4. The regression coefficient was found to be 0.994 with slope value 0.138 and the Y intercept value 0.009 in methanol. The results indicate that there is a linear relationship between concentration (0-10 µg/ml) and absorbance.

Table 1: Calibration Curve of Rosuvastatin Calcium in Phosphate Buffer pH 7.4

Sr. No	Concentration (µg/mL)	Absorbance
1	0	0
2	2	0.13
3	4	0.34
4	6	0.46
5	8	0.59
6	10	0.72

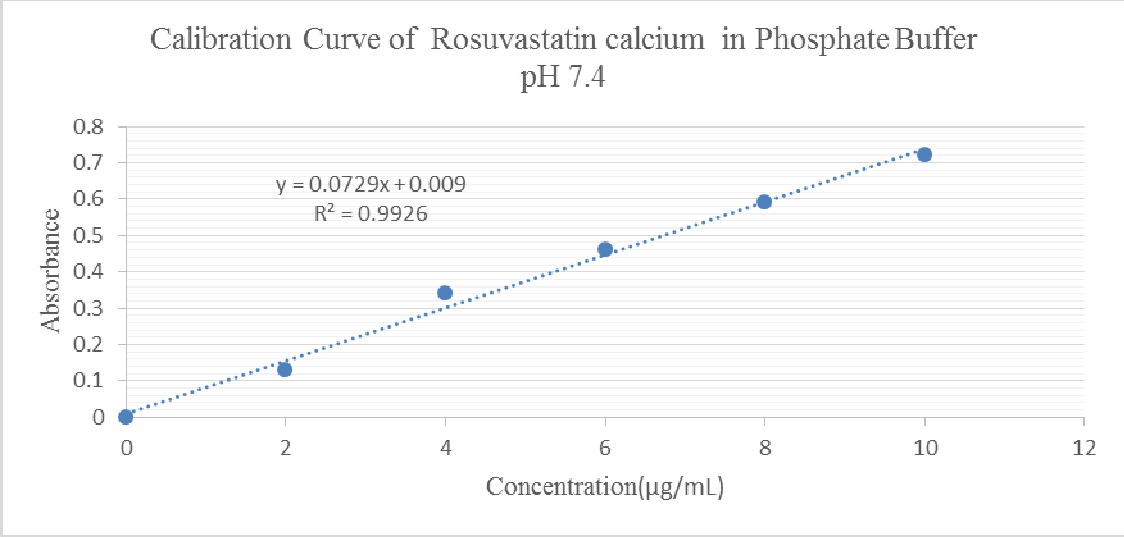

Figure 3: Calibration Curve of Rosuvastatin Calcium in Phosphate Buffer pH 7.4

Table 2: Calibration Curve of Rosuvastatin Calcium in methanol

Sr. No	Concentration (µg/mL)	Absorbance
1	0	0
2	2	0.33
3	4	0.64
4	6	0.90
5	8	1.09
6	10	1.43

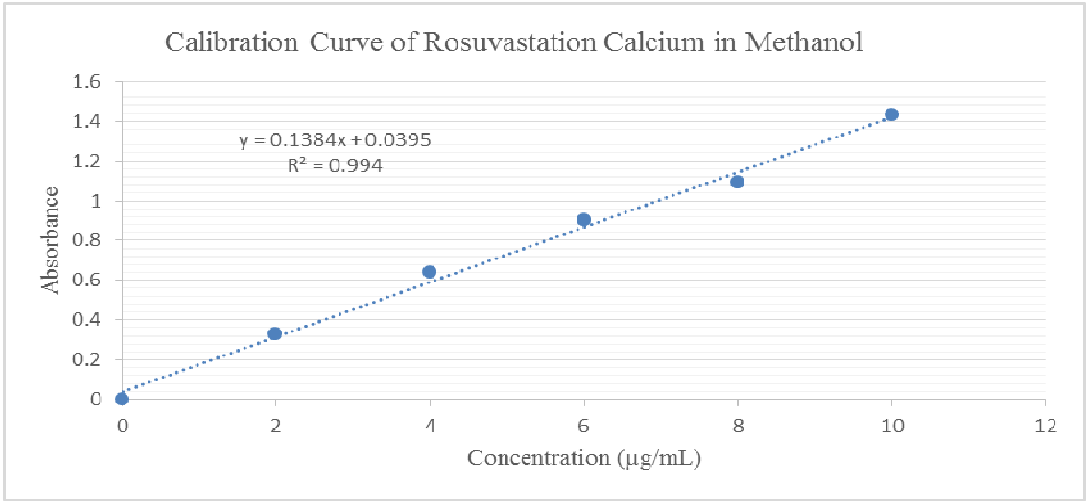

Figure 4: Calibration Curve of Rosuvastatin Calcium in methanol

Screening of excipients by using solubility studies

Table 3: Data for solubility of Rosuvastatin Calcium in various oil phase

Sr. No	Oil Phase	Solubility (mg/ml)
1	Olive Oil	7.68
2	Peppermint oil	12.13
3	Castor Oil	18.06
4	Soybean Oil	9.01
5	Cotton Seed Oil	13.42
6	Castor Oil	19.50
7	Isopropyl Myristate	32.58
8	Dill Oil	15.30
9	Almond Oil	22.71
10	Anise seed Oil	20.24
11	Jojoba Oil	24.82
12	Coconut Oil	15.36

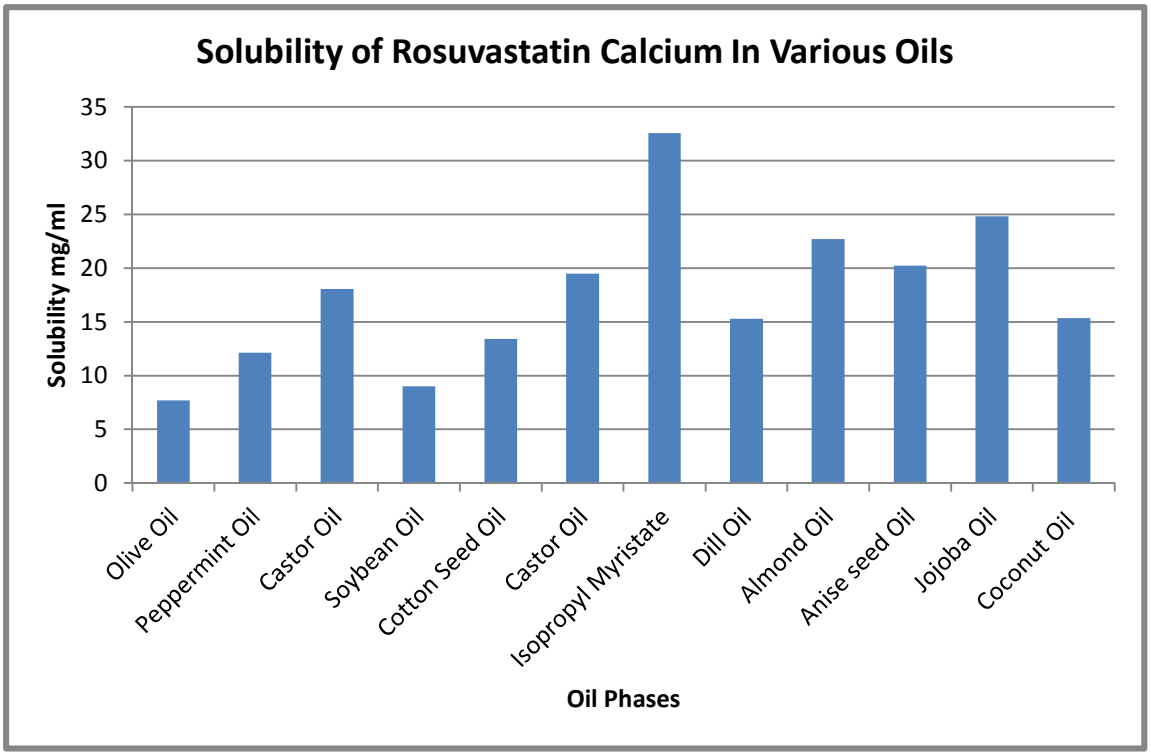

Figure 5: Solubility of Rosuvastatin in Various Oil Phases

Table 4: Data for solubility of Rosuvastatin Calcium in various surfactants

Sr. No	Surfactants	Solubility
1	Tween 20	58.40
2	Tween 40	35.20
3	Tween 80	33.50
4	Span 20	42.35
5	Span 40	25.60

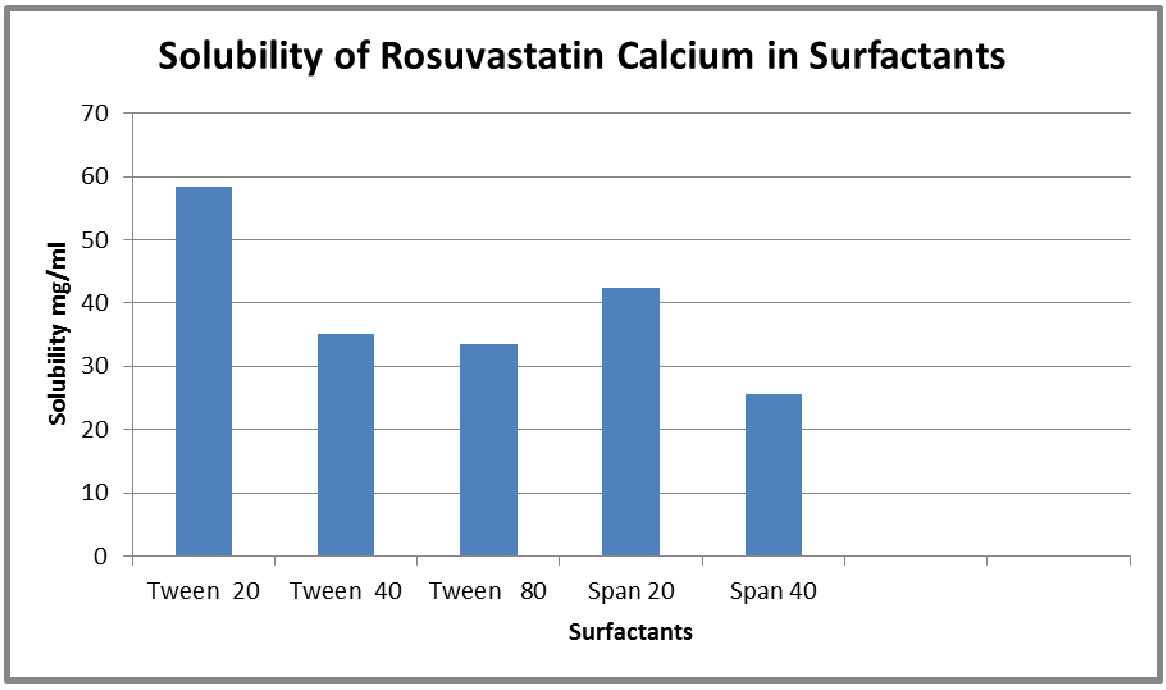

Figure 6: Solubility of Rosuvastatin Calcium in Various Surfactants

Table 5: Data for solubility of Rosuvastatin Calcium in various co-surfactants

Sr. No	Co-Surfactant	Solubility
1	Ethanol	32.42
2	Propylene Glycol	54.77
3	PEG 400	48.14
4	Glycerol	20.48
5	Benzyl alcohol	29.63

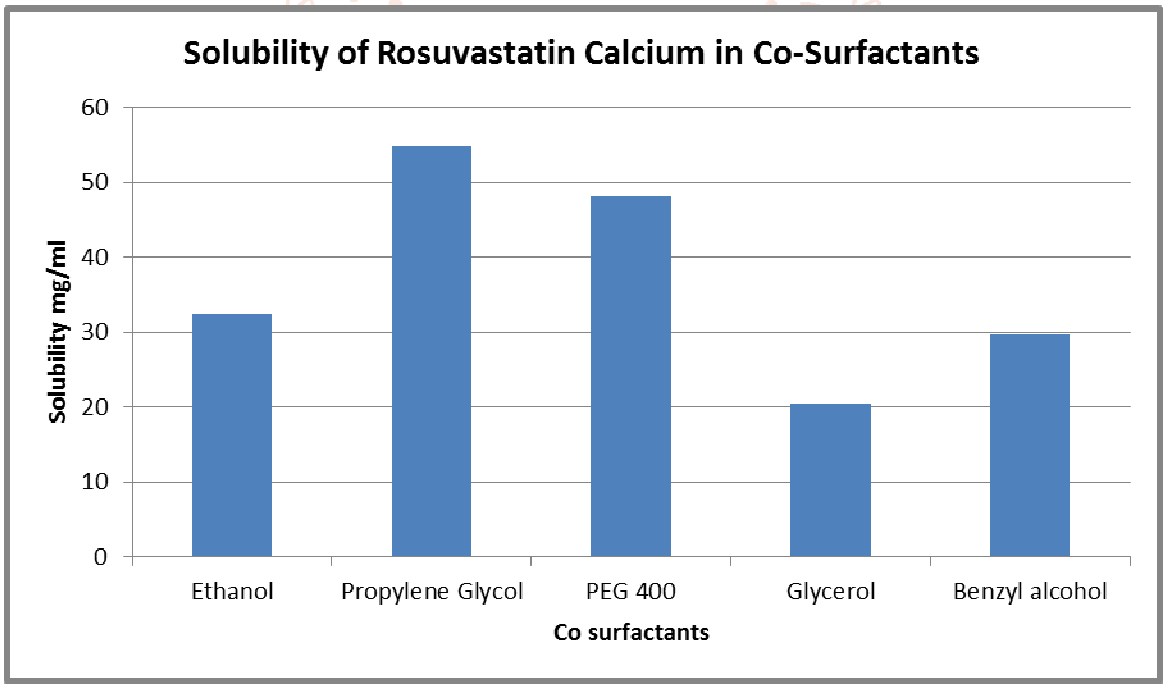

Figure 7: Solubility of Rosuvastatin Calcium in Various Co-Surfactants

Drug Excipient Compatibility Studies

The observed IR peaks of Rosuvastatin Calcium and isopropyl myristate matches with the reported peaks which are shown in the Table.

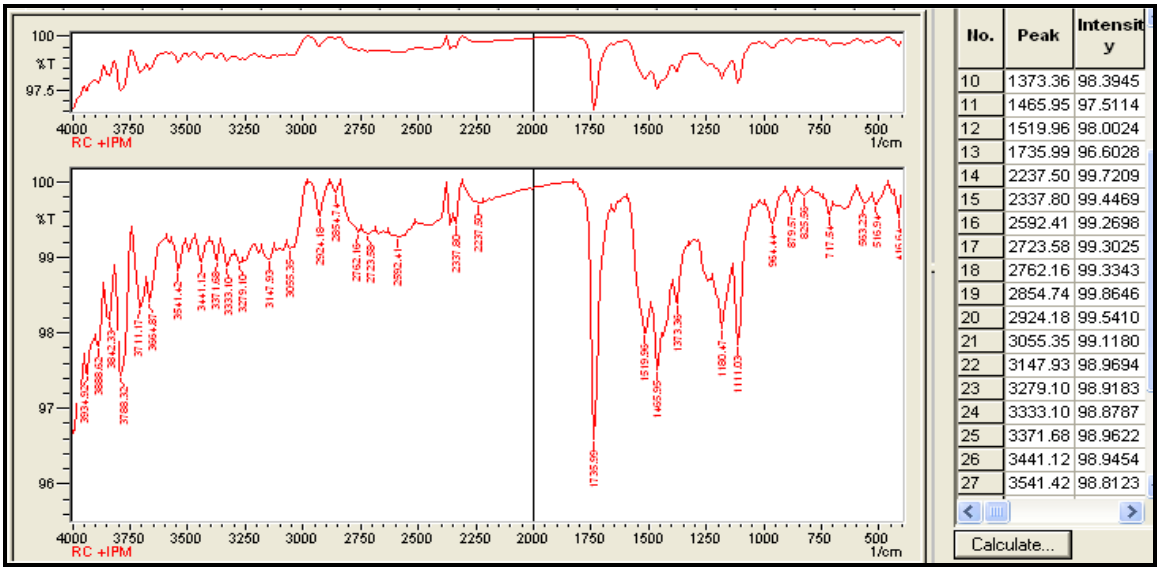

Figure 8: IR Spectra of Rosuvastatin Calcium and isopropyl myristate

Table 6: Interpretation of IR Spectra of Rosuvastatin Calcium and Isopropyl myristate

Group	Wave Number	Absorption Range cm^{-1}
N-H stretching	3541.42	3500
O-H stretching	3371.68	3550-3200
C-H stretching Phenol	2924.18	3000-2500
C-H stretching Alkane	2854.74	3000-2840
C-H stretching Aldehyde	2337.80	2830-2695
C-O stretching	1373.36	1310-1250

The observed IR peaks of Rosuvastatin Calcium and Tween 20 matches with the reported peaks which are shown in the Table

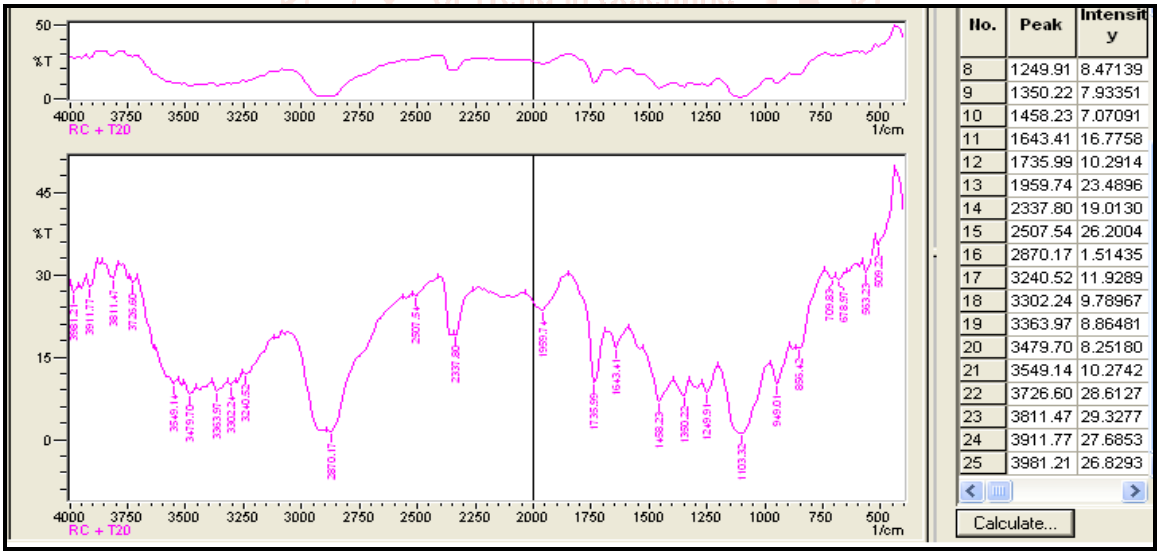

Figure 9: IR Spectra of Rosuvastatin Calcium and Tween 20

Table 7: Interpretation of IR Spectra of Rosuvastatin Calcium and Tween 20

Group	Observed Wave Number	Absorption Range cm^{-1}
N-H stretching	3549.14	3500
O-H stretching	3479.70	3550-3200
C-H stretching Phenol	2507.46	3000-2500
C-H stretching Alkane	2870.17	3000-2840
C-H stretching Aldehyde	2337.80	2830-2695
C=O stretching Ester	1735.99	1750-1735

The observed IR peaks of Rosuvastatin Calcium and propylene glycol matches with the reported peaks which are shown in the Table.

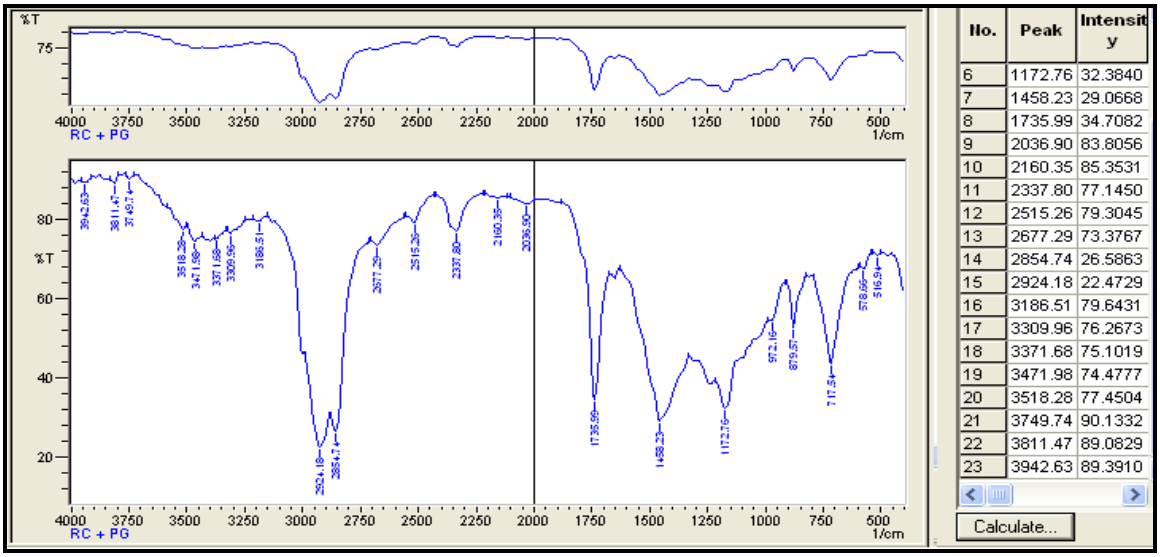

Figure 10: IR Spectra of Rosuvastatin Calcium and Propylene Glycol

Table 8: Interpretation of IR Spectra of Rosuvastatin Calcium and Propylene Glycol.

Group	Observed Wave Number	Absorption Range cm^{-1}
N-H stretching	3518.28	3500
O-H stretching	3471.98	3550-3200
C-H stretching Phenol	2924.18	3000-2500
C-H stretching Alkane	2677.29	3000-2840
C-H stretching Aldehyde	2854.78	2830-2695
O-H bending Alcohol	1458.23	1420-1330

Construction of Pseudo Ternary Phase Diagram

The consideration for screening formulation of microemulsion usually involves: the formulation composition should be simple, safe, and compatible; it should possess good solubility; a large efficient self-emulsification region which should be found in the pseudo-ternary phase diagram, and have efficient droplet size after forming micro-emulsion. Thus, pseudo-ternary phase diagrams were constructed to identify the emulsifying regions with maximum drug loading and to optimize the concentration of oil, surfactant and co-surfactant in the microemulsion formulations and to obtain transparent and stable O/W micro-emulsions.

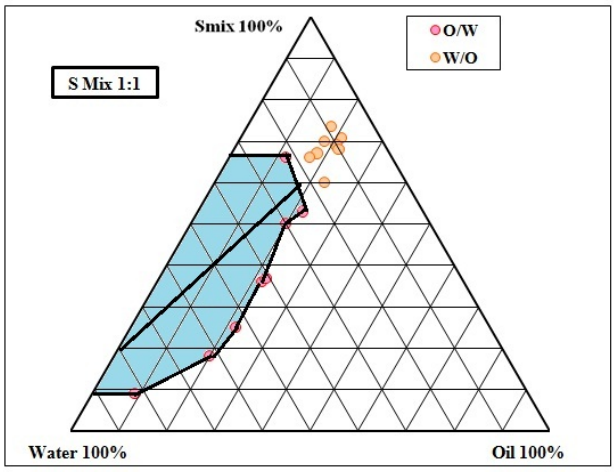

Figure 11: A Smix 1:1

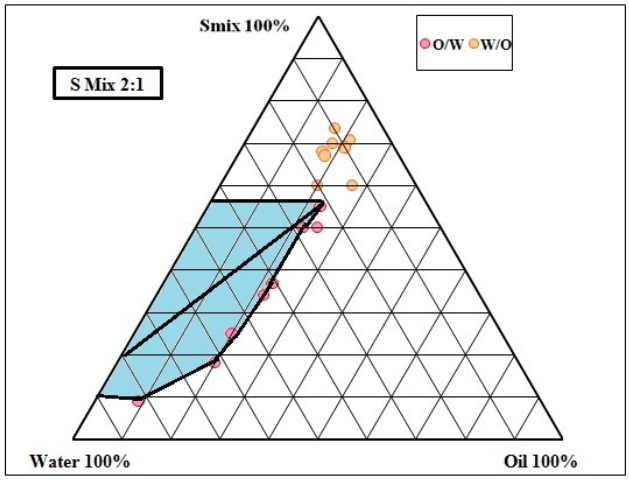

Figure 12: B Smix 2:1

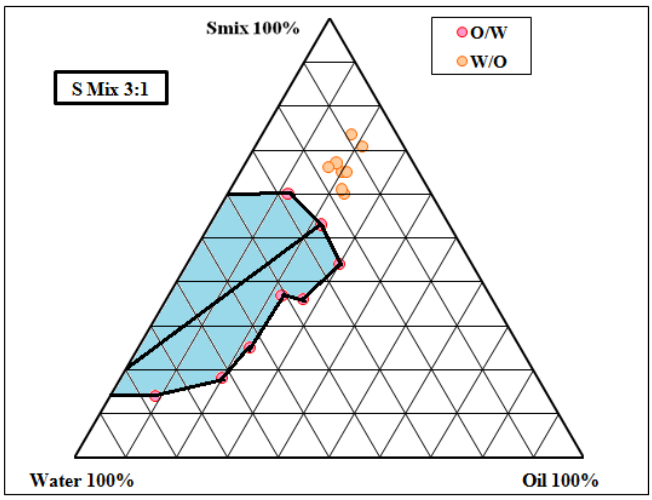

Figure 13: C Smix 3:1

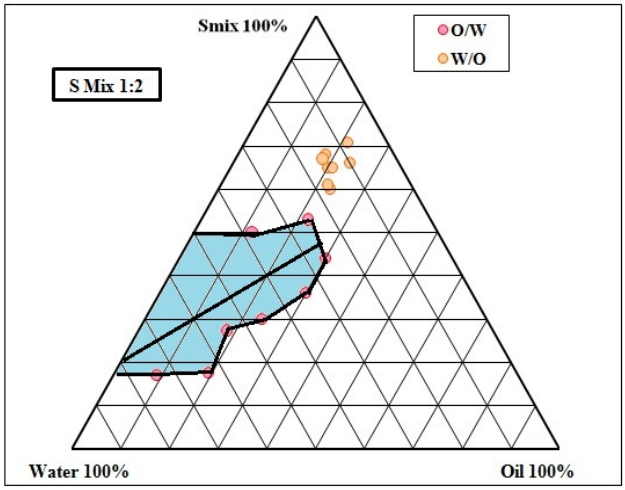

Figure 14: D Smix 1:2

Table 9: Formulations obtained from pseudo ternary phase diagram

Sr. No	Formulations	Oil Phase%	Smix%	Water %
Group 1 S_{mix}=1:1				
1	M1	5	25	70
2	M2	6	32	62
3	M3	10	40	50
4	M4	15	50	35
5	M5	17	60	23
Group 2 S_{mix}=2:1				
6	M1	4	24	72
7	M2	8	32	60
8	M3	12	38	50
9	M4	17	43	40
10	M5	20	50	30
Group 3 S_{mix}=3:1				
11	M1	5	25	70
12	M2	8	32	60
13	M3	12	38	50
14	M4	15	45	40
15	M5	20	50	30
Group 4 S_{mix}=1:2				
16	M1	5	25	70
17	M2	10	30	60
18	M3	15	35	50
19	M4	20	40	40
20	M5	25	45	30

Thermodynamic Stability Studies

Microemulsions are thermodynamically stable systems and are formed at a particular concentration of oil, surfactant and water, with no phase separation, creaming or cracking. It is the thermostability which differentiates nano or microemulsion from emulsions that have kinetic stability and will eventually phase separate. Thus, the selected formulations were subjected to different thermodynamic stability testing by using heating cooling cycle, centrifugation and freeze thaw cycle stress tests. Those formulations, which passed thermodynamic stability tests, were taken for dispersibility test. Thus it was concluded that the efficiency of surfactant and co-surfactant mixture was unaffected after exposing to extreme conditions.

Table 10: Thermodynamic stability test of different formulations selected from Group 1

Group 1 (Fig. A) S_{mix} ratio (S:CoS) 1:1	Percentage w/w of different components in formulation					Inference
Formulations	Oil	S_{mix}	H/C	Cent.	Freez. Tha.	
M1	5	25	X	√	X	Rejected
M2	6	32	√	X	X	Rejected
M3	10	40	√	X	√	Rejected
M4	15	50	X	√	X	Rejected
M5	17	60	√	√	X	Rejected

Table 11: Thermodynamic stability test of different formulations selected from Group 2

Group 2 (Fig. B) S_{mix} ratio (S:CoS) 2:1	Percentage w/w of different components in formulation					Inference
Formulations	Oil	S_{mix}	H/C	Cent.	Freez. Tha.	
M1	4	24	X	√	X	Rejected
M2	8	32	√	√	√	Selected
M3	12	38	√	X	X	Rejected
M4	17	43	√	√	√	Selected
M5	20	50	X	√	X	Rejected

Table 12: Thermodynamic stability test of different formulations selected from Group 3

Group 3 (Fig. C) S_{mix} ratio (S:CoS) 3:1	Percentage w/w of different components in formulation					Inference
Formulations	Oil	S_{mix}	H/C	Cent.	Freez. Tha.	
M1	5	25	X	√	X	Rejected
M2	8	32	X	√	X	Rejected
M3	12	38	√	√	√	Selected
M4	17	43	√	X	X	Rejected
M5	20	50	√	√	√	Selected

Table 13: Thermodynamic stability test of different formulations selected from Group 4

Group 4 (Fig. D) S_{mix} ratio (S:CoS) 1:2	Percentage w/w of different components in formulation					Inference
Formulations	Oil	S_{mix}	H/C	Cent.	Freez. Tha.	
M1	5	25	X	√	X	Rejected
M2	10	30	X	√	√	Rejected
M3	15	35	√	X	X	Rejected
M4	20	40	√	X	√	Rejected
M5	25	45	X	√	X	Rejected

Appearance

By visually inspecting the formulation under light alternately against white and black backgrounds for the existence of turbidity, the appearance was ascertained. When compared to pure water, the transparency of microemulsions was measured in terms of percent Transmittance. Using distilled water to dilute a given amount of the formulation 100 times, the percent transmittance was calculated using a UV-visible spectrophotometer.

Percentage Drug Content

The drug content of all formulations ranged between 95.63 ±0.036 to 98.54±0.021% and passed uniformity of content. The drugs content of M5 formulation was found to be 98.54% while other formulation drug content found less than 97% so it was concluded that M5 formulation have more drug content as compare to others.

Table 14: Percentage Drug Content

Evaluation Parameters	Group 2 Smix (2:1)		Group 3 Smix(3:1)	
	M2	M4	M3	M5
Drug Content (mg/ml)	96.87±0.042	95.63±0.036	97.01±0.023	98.54±0.021

Particle size analysis & Zeta-potential analysis

The rate and extent of drug release and absorption could be dependent on the globule size. From the results of pseudo ternary phase diagram, formulations M2, M3, M4 and M5 were further characterized for measurement of

particle size and zeta potential. Also, it has been reported that the smaller particle size of the emulsion droplets may lead to more rapid absorption as well as enhance the bioavailability of the formulation.

Table 15: Determination of Particle size analysis & Zeta-potential analysis

Sr. No	Evaluation Parameters	Group 2 Smix (2:1)		Group 3 Smix (3:1)	
		M2	M4	M3	M5
1	Mean Particle Size (nm)	45.5	56.4	170.01	122.4
2	Zeta potential (mV)	-12.6	-12.3	-17.5	-22.8

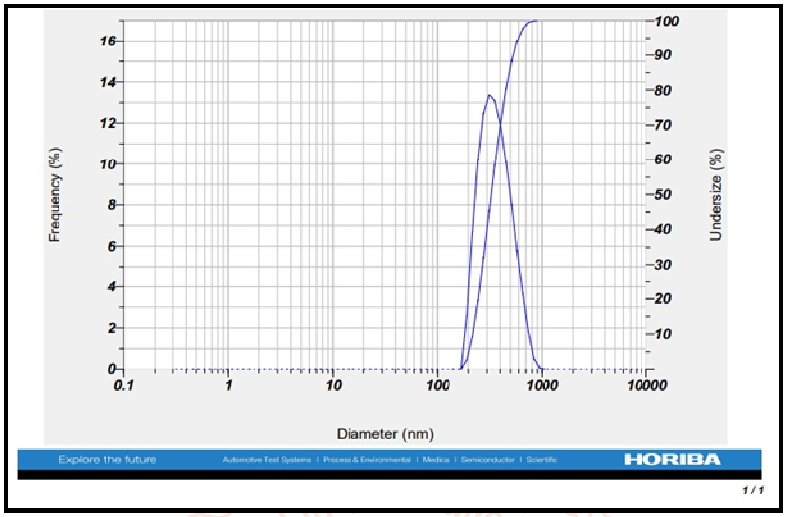

Figure 15: Particle Size Analysis of formulation M2

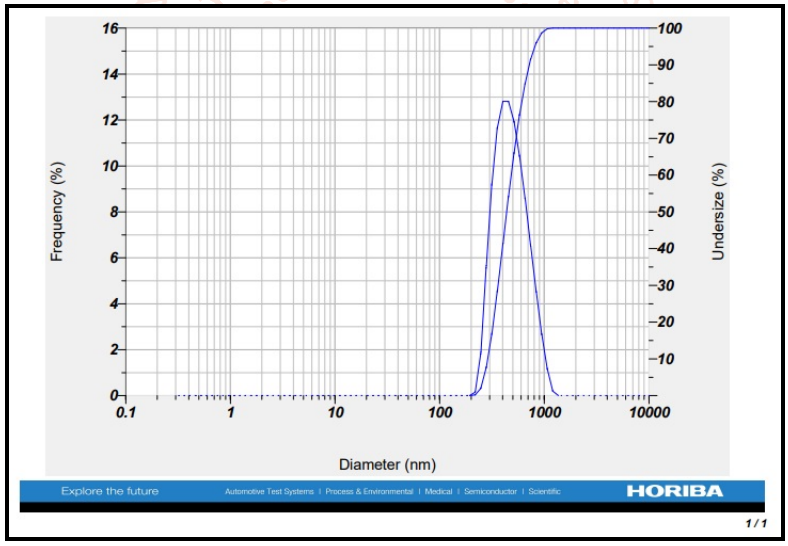

Figure 16: Particle Size Analysis of formulation M3

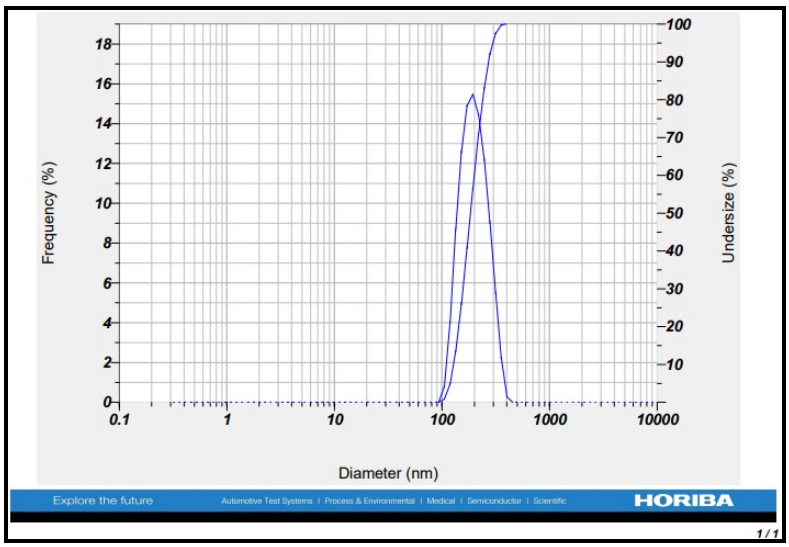

Figure 17: Particle Size Analysis of formulation M4

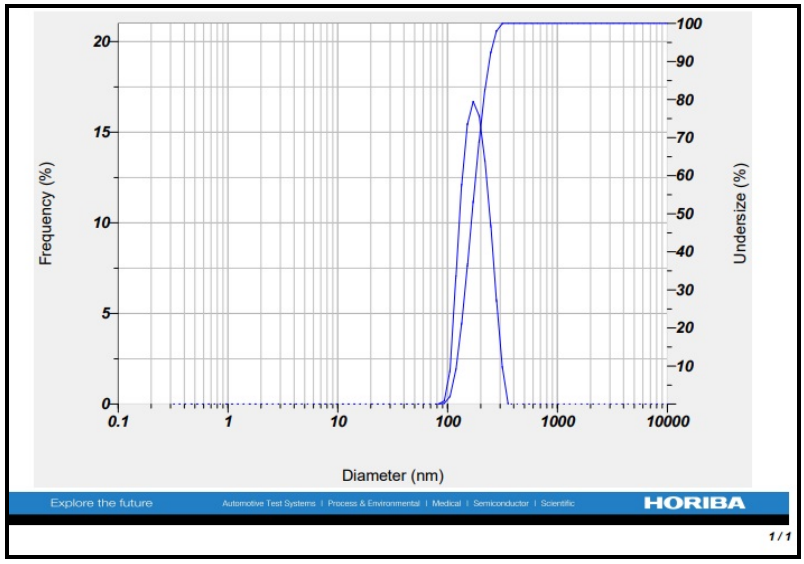

Figure 18: Particle Size Analysis of formulation M5

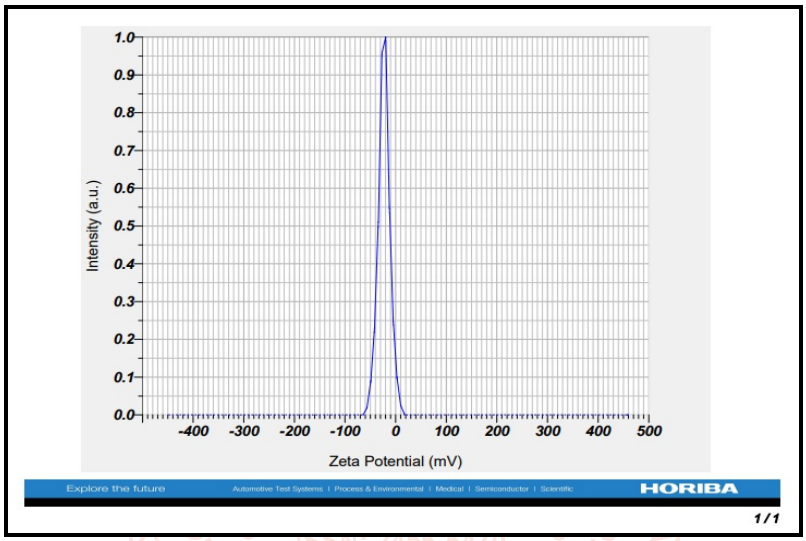

Figure 19: Zeta Potential Analysis of formulation M2

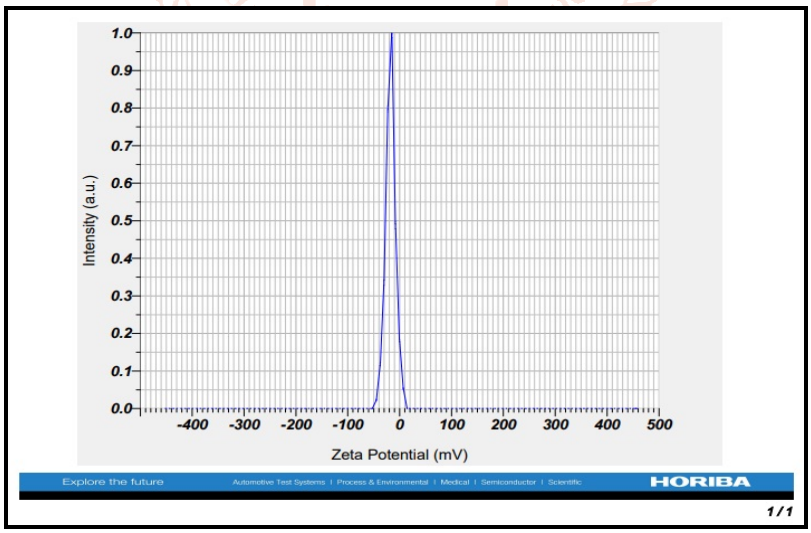

Figure 20: Zeta Potential Analysis of formulation M3

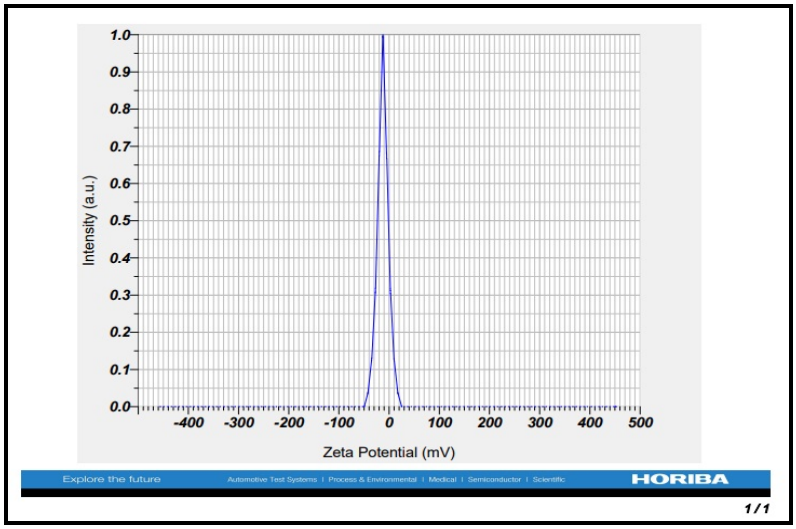

Figure 21: Zeta Potential Analysis of formulation M4

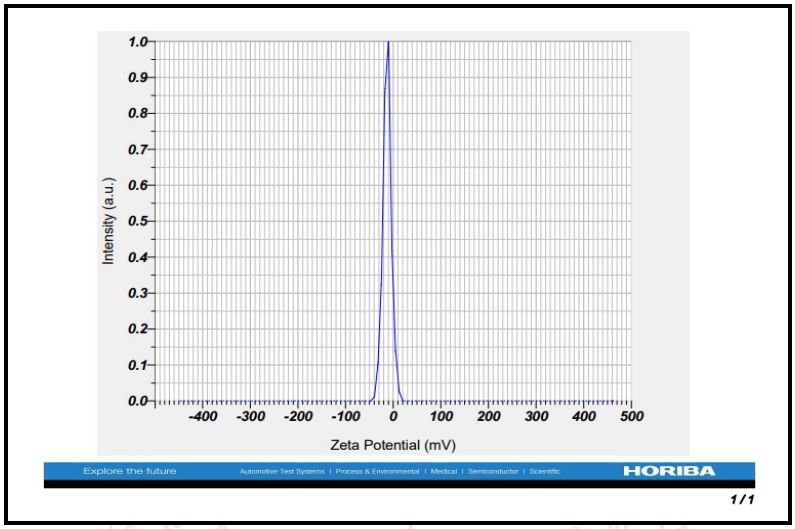

Figure 22: Zeta Potential Analysis of formulation M5

In-vitro drug release study

The in-vitro release of RC-Microemulsion was examined for optimized formulation M3 and M5 was performed. It determines that percentage drug release of M5 formulation is more than M3 formulation. It can be concluded that the developed microemulsion M5 have great potential for transdermal drug delivery.

Table 16: Data for in vitro drug release study

Sr. No	Time (hr)	M3	M5
1	0	0	0
2	0.5	14.29 ±0.99	17.35±1.48
3	1	15.95 ±1.39	20.25 ±1.81
4	1.5	18.32 ±1.54	21.45 ±1.66
5	2	23.22 ±1.75	25.80 ±1.40
6	3	29.53 ±2.07	31.32 ±1.94
7	4	36.12 ±2.25	37.73 ±1.95
8	5	42.13 ±3.37	45.5 ±3.57
9	6	45.46 ±2.25	50.21 ±4.47
10	7	52.14 ±3.00	55.3 ±3.81
11	8	54.11±4.56	56.95±3.79
12	9	56.32±3.56	63.22±4.51
13	10	56.8±4.44	65.87±3.86
14	11	57.11±5.51	66.22±4.71
15	12	57.98±5.49	67.58±4.58
16	22	58.22±5.58	68.33±5.08
17	24	59.56±5.04	69.56±5.23

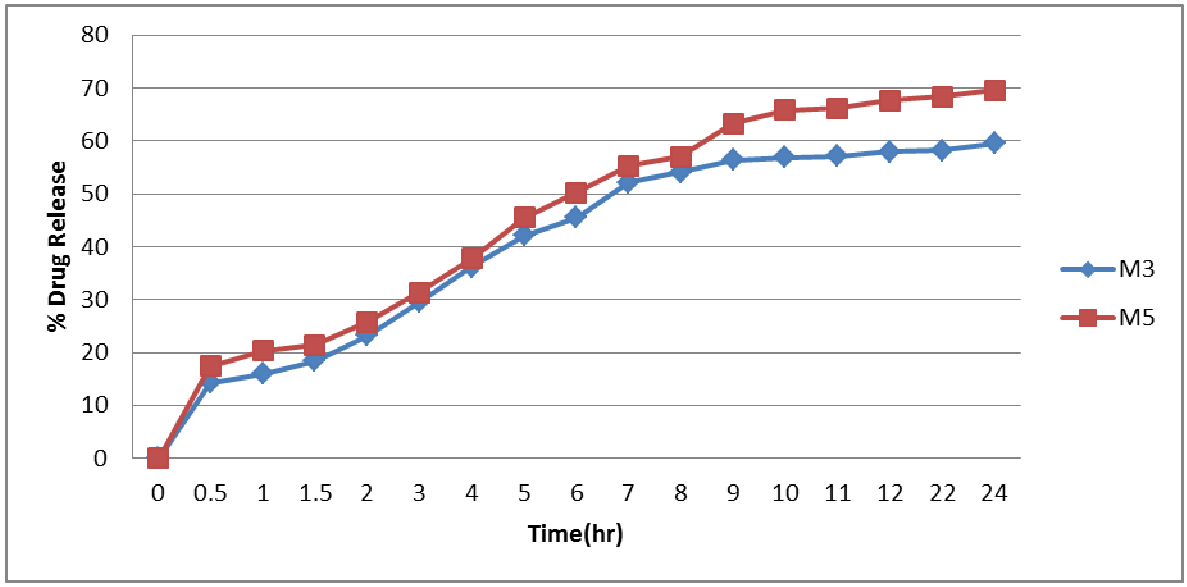

Figure 23: In-vitro drug release study

CONCLUSION

Microemulsions are commonly referred to complex liquids. In many cases this complex liquids can be turned into an advantages and the systems turn out to be simpler than the simple liquids. Microemulsions can be successfully bypass the hepatic first pass metabolism on transdermal application.

In this present study, Rosuvastatin Calcium microemulsions were successfully prepared and evaluated for different parameters such as particle size, zeta potential, in-vitro drug release and stability studies. The optimized microemulsion (M5) formulation consist of oil phase isopropyl myristate (20%), surfactant tween 20 (37.5%) and cosurfactant propylene glycol (12.5%) with zeta potential of -22.8 mV and particle size 122.4 nm. Stability data indicated stable formulation.

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