GSC Biological and Pharmaceutical Sciences
eISSN: 2581-3250 CODEN (USA): GBPSC2
Cross Ref DOI: 10.30574/gscbps
Journal homepage: https://gsconlinepress.com/journals/gscbps/

(RESEARCH ARTICLE)

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Development of rosuvastatin calcium nano-carrier patches by central composite design

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GSC Biological and Pharmaceutical Sciences, 2024, 27(03), 123–133

Publication history: Received on 07 November 2023; revised on 13 June 2024; accepted on 16 June 2024

Article DOI: https://doi.org/10.30574/gscbps.2024.27.3.0528

Abstract

Rosuvastatin (RS) is a statin medication used to lower cholesterol levels and reduce the risk of cardiovascular diseases. RS works by inhibiting an enzyme called HMG-CoA reductase, which helps decrease LDL cholesterol and increase HDL cholesterol. The typical dose range for rosuvastatin is 5-40 mg once daily, with the starting dose usually being 10 mg. However, the dosage may vary depending on the patient's cholesterol levels and individual response to the medication. Rosuvastatin has a half-life of approximately 19 hours and undergoes metabolism mainly in the liver. The aim of the study was to develop a transdermal patch containing Rosuvastatin in the form of niosomes (NP). The Rosuvastatin niosomes were prepared using the solvent casting method, and the optimized formulation showed a particle size of 211.6 nm with a polydispersity index (PDI) of 0.197. The zeta potential of the niosomes was found to be -44.7. These results indicate that the developed formulation has a uniform particle size distribution and a stable zeta potential, which are important factors for the effective delivery of Rosuvastatin through a transdermal patch. Invitro diffusion studies of the optimized patch RN-T6 released upto 24 hours with %CDR of 98.98% and the drug diffusion data is fitted into various mathematical models and RN-T6 followed zero order drug release kinetics and the diffusion mechanism was non-fickian diffusion.

Keywords: Rosuvastatin; Niosomal patch; Particle size; Zeta potential; Poly dispersibility index; Design of experiment

1. Introduction

Rosuvastatin, is a widely prescribed medication for cardiovascular diseases that belongs to the class of drugs called statins. It is primarily used to lower high levels of LDL (bad) cholesterol and triglycerides in the blood while simultaneously increasing levels of HDL (good) cholesterol[1,2]. By inhibiting an enzyme called HMG-CoA reductase, rosuvastatin helps to decrease cholesterol production in the liver and promotes the uptake and clearance of LDL cholesterol from the bloodstream. This medication is commonly prescribed to patients with hypercholesterolemia, a condition characterized by increased levels of cholesterol, as well as individuals at high risk of cardiovascular diseases such as heart attack and stroke. Rosuvastatin not only improves lipid profiles but also offers potential benefits in other areas, including reducing inflammation and stabilizing plaque in the arteries[3,4].

Niosomes are non-ionic surfactant vesicles obtained by hydrating mixture of cholesterol and nonionic surfactants. Niosomes play an increasingly important role in drug delivery as they can reduce toxicity and modify pharmacokinetic and bio-availability[5,6]. It can act as drug containing reservoirs and the modification of the vesicular compositions or surface properties can adjust the drug release rate. The RS niosomes were prepared by thin film hydration technique and the optimized niosomes were formulated into transdermal patch by using various polymers for controlled release of the drug[7,8].

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Rosuvastatin niosomal patches offer numerous advantages for the delivery of the medication. Firstly, the niosomal formulation improves the stability of Rosuvastatin, protecting it from degradation and maintaining its effectiveness over a longer period. These patches also enhance the penetration of Rosuvastatin through the skin, ensuring efficient drug delivery and potentially improving therapeutic outcomes[9,10]. Additionally, niosomal patches enable controlled release of the medication, providing a sustained and consistent delivery of Rosuvastatin. This controlled release helps to improve patient compliance and minimize fluctuations in drug concentration in the bloodstream[11,12].

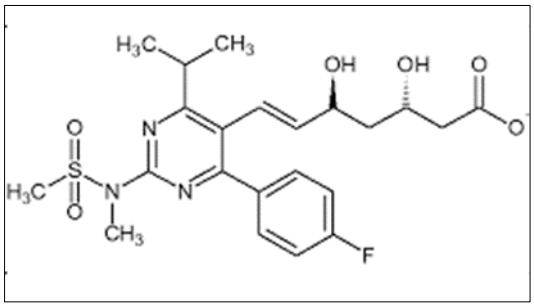

Figure 1 Molecular structure of Rosuvastatin

2. Materials and Methods

2.1. Materials

Rosuvastatin obtained from Aurobindo pvt. Ltd., Span 60, Span 80, Tween 40, Tween 60, Tween 80, Cholesterol, soy lecithin, HPMC E5, HPMC 5cps, HPMC 15cps, Carbopol 734, sodium alginate, methanol, chloroform, PEG 200, glycerine, Labrasol ALF, Transcutol, Methanol (Merck), Orthophosphoric acid etc. All the chemicals were of analytical grade.

2.2. Methods

2.2.1. Analytical Method Development

Construction of Standard Graph of Rosuvastatin calcium

A 100 mg quantity of Rosuvastatin calcium was accurately weighed and transferred into a 100 mL volumetric flask. To dissolve the drug, 20 mL of methanol was added, and the volume was made up to 100 mL using distilled water. This resulting solution had a concentration of 1 mg/mL and was labeled as 'stock'. From the stock solution, 10 mL was taken and diluted to 100 mL using methanol, resulting in a solution with a concentration of 100 µg/mL. Various dilutions of Rosuvastatin calcium (ranging from 2 to 10 µg/mL) were prepared using this second solution. The prepared solutions were then analyzed using a UV spectrophotometer, scanning the absorbance between 200-800 nm when compared to a blank solution[13,14] .

2.2.2. Solubility of the Rosuvastatin calcium in various Excipients

The solubility of Rosuvastatin was resolved in different surfactants. Excess amount of formulation was added to 1 gram of each excipient in various cap vials. The mixture was made to cyclomix immediately using cyclo-mixer for 2 min which facilitates drug solubilisation. Cap vials were stirred in a water bath at 40-50°c for 5 min and then it is allowed to reach equilibrium at room temperature in an isothermal rotary shaker at 100rpm speed for 72hours. Each vial was centrifuged at speed of 3,000 rpm for 15 min using a centrifuge (Remi Equipment) followed by removal of the undissolved drug by separating the supernatant liquid and aliquots of supernatant fluid was drawn utilizing micropipette which are suitably diluted with methanol. The drug concentration in various excipients was obtained spectrophotometrically via a validated UV method at λmax 257 nm[13-17]. The surfactants with higher solubility are used in formulation of Niosomes.

2.2.3. Drug excipients compatibility studies

Study of drug-excipient compatibility is an important phase in the early stage of drug development.it defines the potential interactions between API and the excipients in the formulation. These studies are helpful in the selection of excipients, outline the stability of drug and distinguish the degraded excipients. FTIR studies were performed by screening the samples in the scope of 400-4000 cm-1. The absorption peaks which were observed for the pure drug was

compared with the optimized formulation and excipients. The characteristic absorption peaks of Drug were observed in the combination spectrum which shows that the Drug and excipients are compatible with each other[13-17].

2.2.4. Design of experiments

Design of experiments is used for optimization of niosomes and response surface graphs are shown in Fig-8. Central Composite Design (CCD) is a popular experimental design technique used to study the relationship between multiple variables and a response variable of interest. It is a type of response surface methodology (RSM) that allows for modeling and optimization of processes. The main objective of Central Composite Design is to determine the relationship between independent variables (factors) and a response variable while considering both linear and nonlinear effects. Each factor is set at various levels to cover a broad range of values. The factor levels can be high (+1), low (-1), or intermediate (0). The actual values for each factor level depend on the experimental system and the units of the factors. Quadratic model is used with 15 runs[23-26].

2.2.5. Preparation of Rosuvastatin Niosomes

The surfactants with highest solubility of RS were choose for formulating Niosomes. Span 60, Span 80, Tween 40, Tween 60 and Tween 80 were chosen for the study. The surfactants were placed in a round bottomed flask. The solvent system is then added to the mixture and the ingredients were dissolved in the sol-vent (Chloroform: methanol) by hand shaking. The flask was attached to a rotary evaporator and immersed in water bath maintained at 60˚C, rotated at 100rpm for 45min. Formation of thin film at the bottom was observed. The thin film is hydrated using 6.8pH buffer. The resultant solution was sonicated in Bath sonicator for 10mins. The niosomal dispersion formulations were shown in the Table-1 with various surfactants and their composition[13-17]. The Rosuvastatin niosomes containing Tween 40 was selected for DoE for further optimization.

Table 1 Composition of Rosuvastatin Niosomal Dispersion

Formulation code	Surfactant Name	Rosuvastatin (mg)	Surfactant (mg)	Cholesterol (mg)	Soya Lecithin (mg)	Solvent (Chloroform: Methanol) (ml)	Buffer Solution (ml)
RN1	Span 60	200	100	100	50	2:1	4ml
RN2	Span 60	200	200	100	50	2:1	4ml
RN3	Span 80	200	100	100	50	2:1	4ml
RN4	Span 80	200	200	100	50	2:1	4ml
RN5	Tween 40	200	100	100	50	2:1	4ml
RN6	Tween 40	200	200	100	50	2:1	4ml
RN7	Tween 60	200	100	100	50	2:1	4ml
RN8	Tween 60	200	200	100	50	2:1	4ml

2.2.6. Characterization of Niosomes

Particle size, PDI and Zeta potential

Rosuvastatin niosomal sample was diluted to 100ml of water and was blended utilizing magnetic stirrer. The globule size and zeta potential analysis were measured after an hour by Dynamic Light Scattering (DLS) spectroscopy utilizing an instrument named Zetasizer Nano ZS 90 Version 7.10 (Malvern Instruments). The sizing of the niosomes were performed by placing a disposable sizing cuvette and zeta potential analysis was done by utilizing an electrophoretic cell with a point of recognition of 90° measurement [13-17]. The results were shown in Table-2.

Table 2 Characterization of Rosuvastatin Niosomes

Formulation code	Vesicle Size(nm)	PDI	Zeta Potential (mv)
RN1	466.9	0.405	-6.39
RN2	384.1	0.099	-18.5
RN3	767.7	0.931	-20.6
RN4	360.4	0.236	-22.7
RN5	241.9	0.273	-30.1
RN6	264.5	0.475	-34.3
RN7	753.5	0.781	-25.9
RN8	588.7	0.053	-29.1

Table 3 Optimization of Rosuvastatin Niosomal Dispersion with DoE

	Factor 1	Factor 2	Factor 3	Response 1
Run	**A: Tween 80 concentration**	**B: Temperature of hydration**	**C: Time of Hydration**	**Entrapment efficiency**
	Mg	**Celsius**	**Min**	**%**
RN-R1	100	55	20	72.32
RN-R2	200	65	15	91.24
RN-R3	100	65	15	73.13
RN-R4	200	75	20	88.91
RN-R5	100	55	10	63.87
RN-R6	200	55	10	79.56
RN-R7	200	55	20	89.06
RN-R8	150	75	15	72.25
RN-R9	150	65	10	75.84
RN-R10	150	65	20	76.57
RN-R11	100	75	20	66.89
RN-R12	150	55	15	71.38
RN-R13	100	75	10	66.73
RN-R14	150	65	15	76.69
RN-R15	200	75	10	87.47

Percentage drug entrapment (PDE)

- Drug loading efficiency

Selected formulations were evaluated for the determination of drug content. In this method 0.1ml of the formulation was diluted with methanol (100ml)[11]. The drug loading efficiency was calculated by the formula given below:

Drug loading efficiency= amount of drug (API) present in the formulation ÷ Initial drug load× 100

The entrapped Rosuvastatin within niosomes was determined after removing the unentrapped drug by dialysis. The dialysis was carried out by taking niosomal dispersion in dialysis bag, which was dipped in a beaker containing 400 ml of PBS with a pH of 7.4 the beaker was placed on a magnetic stirrer run for 4 h with a speed of 80- 120 rpm. Then, the solution inside the receptor compartment was studied for unentrapped drug. The PDE in the niosomes was calculated from the ratio of the difference of the total amount of drug added and the amount of unentrapped drug detected, to the total amount of drug added[13-17]. The results were shown in Table-4.

Table 4 Characterization of Rosuvastatin Niosomes (DoE)

Formulation code	Vesicle Size(nm)	PDI	Zeta Potential (mv)
RN-R2	211.6	0.197	-44.7
RN-R7	239.9	0.264	-39.2

Transmission Electron Microscopy (TEM)

External morphology of prepared niosomal suspension was determined using transmission electron microscopy. Sample of the niosomal dispersion was prepared by placing a drop onto a copper grid. Digital Micrograph and Soft Imaging Viewer software were used to perform the image capture and analysis, including particle size[13-17].

2.2.7. Formulation of RS patch and RS loaded niosomal patch

RS patch and RS loaded niosomal dispersion were shown in tables 5 & 7 and characterized. The prepared Niosomal formulations were incorporated into transdermal patch by solvent casting method using aluminium foil as a backing membrane. The various polymer combinations were weighed accurately and mixed in Chloroform: Methanol mixture stirred for 30min. Finally, the plasticizer (PEG-400) was dropped into the solution with stirring for more half an hour. Then the solution was kept aside overnight for clearance of all the bubbles. The next day, it was poured onto the Teflon plates set at room temperature and left over until uniformly dried film is obtained[18-22].

Table 5 Formulation table of Rosuvastatin Calcium Transdermal patches by solvent casting method

Formulation Code	RT1	RT2	RT3	RT4	RT5	RT6	RT7	RT8	RT9
Rosuvastatin (mg)	20	20	20	20	20	20	20	20	20
Polymers (mg)									
HPMC E5: HPMC 15 CPS	125	125	125	-	-	-	-	-	-
HPMC 5 CPS: HPMC 15 CPS	-	-	-	125	125	125	-	-	-
Carbopol 734: HPMC 15 CPS	-	-	-	-	-	-	125	125	125
Labrasol ALF	0.05	-	-	0.05	-	-	0.05	-	-
Transcutol	-	0.05	-	-	0.05	-	-	0.05	-
Span 60	-	-	0.05	-	-	0.05	-	-	0.05
Plasticizer (ml)									
PEG 200	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Glycerine	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Solvents (ml)									
Chloroform	3	3	3	3	3	3	3	3	3
Methanol	2	2	2	2	2	2	2	2	2

Table 6 Evaluation of Rosuvastatin Transdermal Patches

Formulation Code	Weight Variation (mg)	Thickness (mm)	% Moisture Content	Folding Endurance	Drug Content	Surface pH
RT1	296±09	0.254±0.16	0.76±0.01	>300	95.50 ± 4.60	6.54 ± 0.06
RT2	271±27	0.261±0.19	0.61±0.03	>300	99.05 ± 8.10	6.39 ± 0.16
RT3	265±76	0.289±0.18	0.34±0.04	>300	98.50 ± 5.40	6.85 ± 0.03
RT4	301±19	0.219±0.12	0.26±0.09	>300	99.70 ± 5.70	6.86 ± 0.13
RT5	287±21	0.362±0.09	0.38±0.04	<300	98.40 ± 3.50	6.37 ± 0.14
RT6	278±34	0.209±0.13	0.63±0.07	<300	91.90 ± 3.90	6.43 ± 0.25
RT7	269±23	0.271±0.09	0.41±0.04	>300	94.60 ± 6.30	6.74 ± 0.16
RT8	289±23	0.223±0.12	0.37±0.03	>300	93.70 ± 2.90	6.69 ± 0.03
RT9	299±56	0.217±0.13	0.48±0.07	>300	95.40 ± 8.70	6.29 ± 0.25

Table 7 Formulation table of Rosuvastatin Niosomal Transdermal patches by solvent casting method

Formulation Code	RNT1	RNT2	RNT3	RNT4	RNT5	RNT6	RNT7	RNT8	RNT9
Rosuvastatin Niosomal Dispersion (ml)	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
Polymers (mg)									
HPMC E5: HPMC 15 CPS	125	125	125	-	-	-	-	-	-
HPMC 5 CPS: HPMC 15 CPS	-	-	-	125	125	125	-	-	-
Carbopol 734: HPMC 15 CPS	-	-	-	-	-	-	125	125	125
Labrasol ALF	0.05	-	-	0.05	-	-	0.05	-	-
Transcutol	-	0.05	-	-	0.05	-	-	0.05	-
Span 60	-	-	0.05	-	-	0.05	-	-	0.05
Plasticizer (ml)									
PEG 200	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Glycerine	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Solvents (ml)									
Chloroform	3	3	3	3	3	3	3	3	3
Methanol	2	2	2	2	2	2	2	2	2

In vitro diffusion studies

The Franz Diffusion cell was used for invitro permeation study. The Franz Diffusion Cell is a simple, reproducible test for measuring the *in vitro* drug release from creams, ointments and gels. The Franz Cell consists of two primary chambers separated by a membrane. The test product is applied to the membrane via the top chamber- donor compartment. The bottom chamber- receptor compartment contains fluid from which samples are taken at regular intervals for analysis. This testing determines the amount of active drug that has permeated the membrane at each time point. The cellophane membrane was mounted on a diffusion cell assembly with an effective diffusion area of 2.303 cm^2. The receptor compartment consisted of a 22.5 ml phosphate buffer at pH 6.8 as the receptor fluid agitated at 100 rpm, and was maintained at 37 ± 0.5°C throughout the experiments. The cumulative amount that permeated across the cellophane membrane was calculated and plotted against time[18-22].

Kinetic analysis of diffusion data

- Drug release kinetic analysis of Rosuvastatin calcium

To determine the drug release from the optimized formulation of Rosuvastatin niosomal patches, various mathematical models were utilized such as zero order, first order, Hixson and crowels, Higuchi model & korsemeyer-peppas model. In zero order kinetic pattern the rate of drug release is independent of its concentration whereas in first order kinetic model the rate of drug release depends on the concentration[23-26].

Table 8 Evaluation of Rosuvastatin Niosomal Transdermal Patches

Formulation Code	Weight Variation (mg)	Thickness (mm)	% Moisture Content	Folding Endurance	Drug Content	Surface pH
RNT1	376 ± 23.2	0.317 ± 0.13	0.57 ± 0.329	>200	90.53 ± 2.54	6.76 ± 0.33
RNT2	347 ± 19.4	0.343 ± 0.11	0.48 ± 0.139	>200	99.14 ± 4.59	6.58 ± 0.13
RNT3	365 ± 47.9	0.372 ± 0.12	0.77 ± 0.049	>200	97.68 ± 3.82	6.90 ± 0.15
RNT4	357 ± 58.1	0.291 ± 0.11	0.67 ± 0.049	>200	96.20 ± 3.60	6.68 ± 0.26
RNT5	366 ± 42.6	0.284 ± 0.10	0.38 ± 0.029	<200	98.14 ± 6.83	6.59 ± 0.63
RNT6	364 ± 21.5	0.312 ± 0.13	0.72 ± 0.029	>200	95.95 ± 1.71	6.73 ± 0.22
RNT7	348 ± 38.7	0.349 ± 0.17	0.97 ± 0.109	>200	97.53 ± 2.43	6.96 ± 0.44
RNT8	370 ± 44.5	0.341 ± 0.12	0.09 ± 0.029	<200	97.15 ± 3.53	6.41 ± 0.74
RNT9	372 ± 31.9	0.317 ± 0.11	0.59 ± 0.099	>200	97.13 ± 4.16	6.53 ± 0.34

Table 9 Drug release Kinetics of optimised Rosuvastatin Transdermal Patch

Formulation code	Zero order	First order	Hixson & Crowells	Higuchi	Korsemeyer-Peppa's equation		Diffusion mechanism
	R^2	R^2	R^2	R^2	R^2	n	
RN-T2	0.9886	0.845	0.8727	0.9302	0.939	1.2439	Non-Fickian diffusion

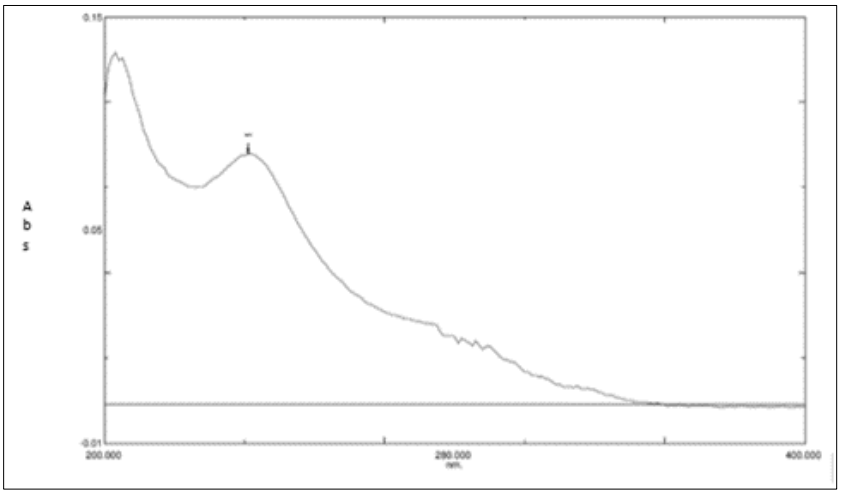

Figure 2 UV-Spectra of Rosuvastatin calcium

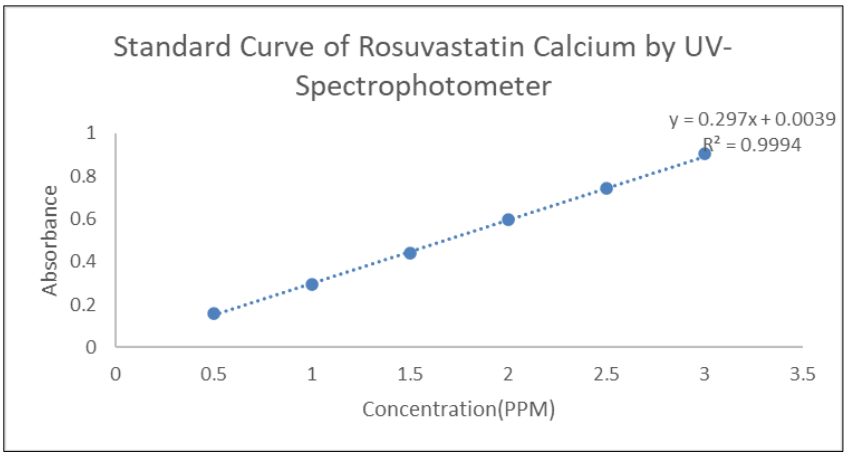

Figure 3 Calibration curve of Rosuvastatin calcium

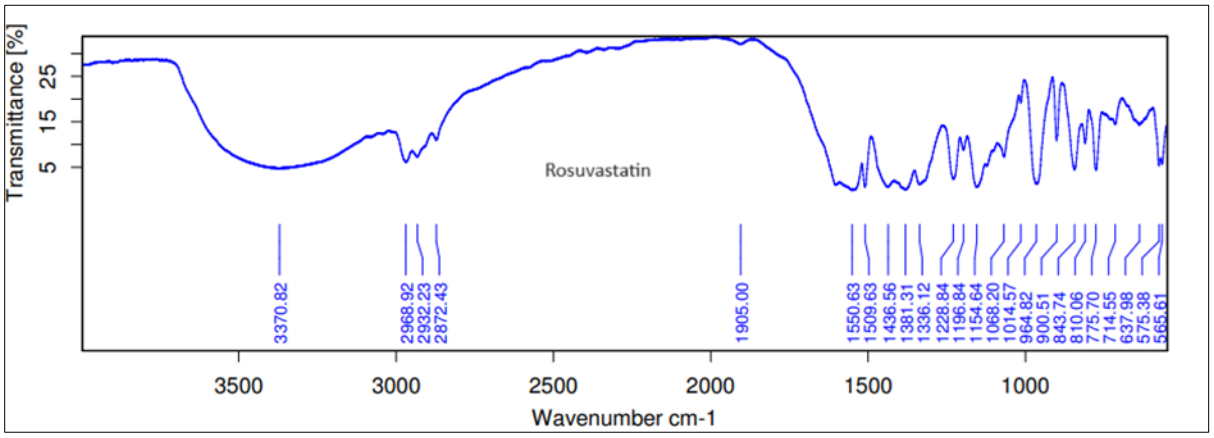

Figure 4 FTIR spectra of Rosuvastatin calcium

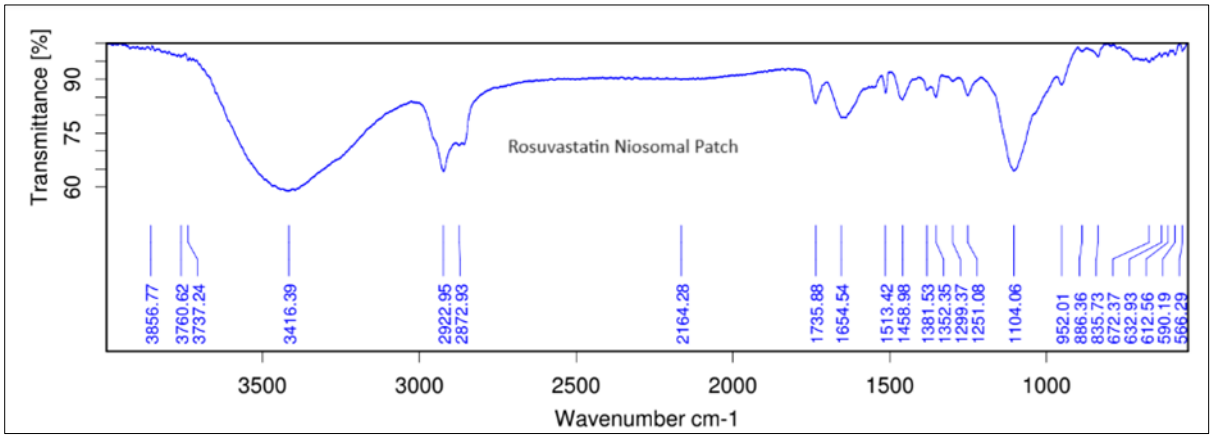

Figure 5 FTIR spectra of RS Niosomal patch

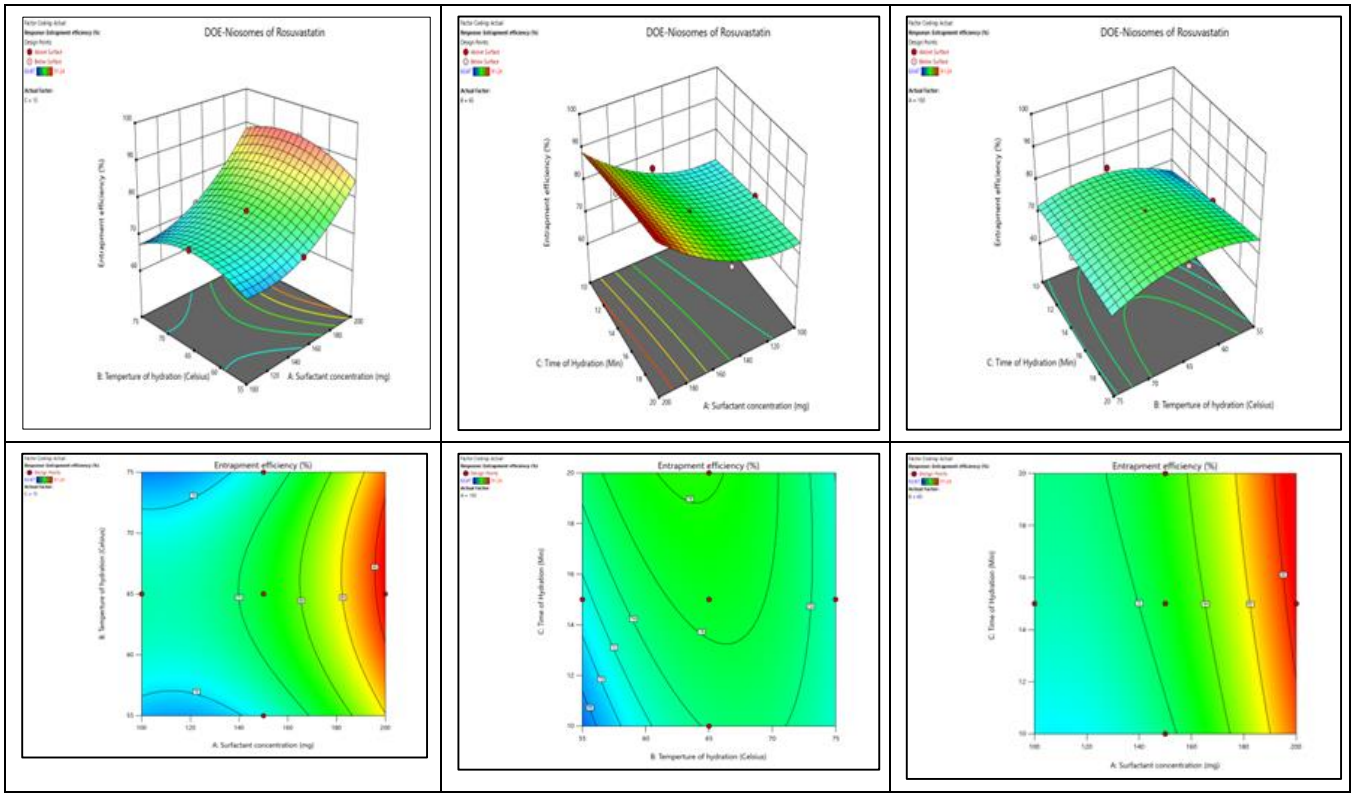

Figure 6 DoE graphs of Rosuvastatin Niosomes

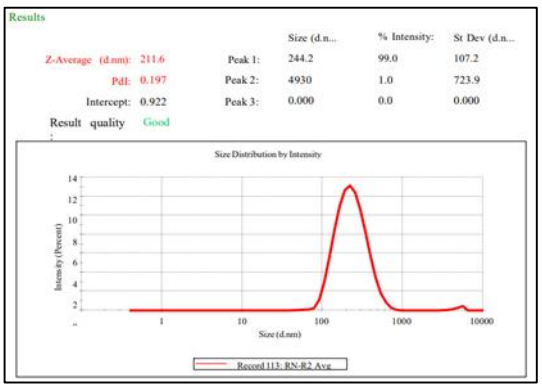

Figure 7 Size and PDI of RN-R2

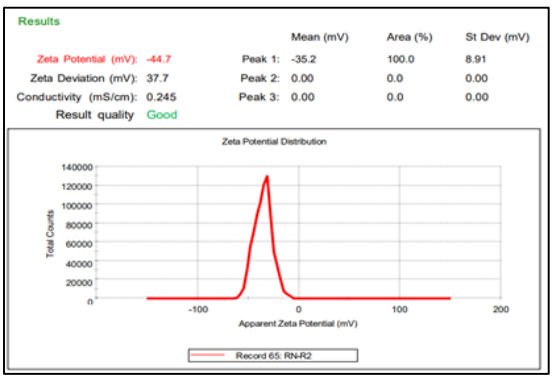

Figure 8 Zeta potential of RN-R2

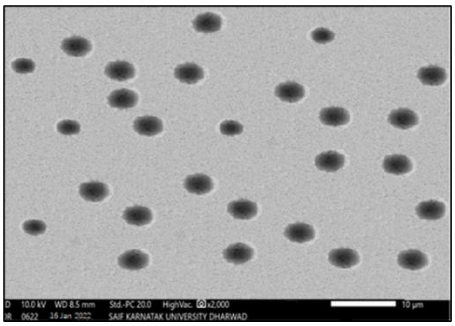

Figure 9 TEM image of RN-R2

3. Discussion

The UV-scan spectrum of Rosuvastain was found to be at 257nm and calibration curve of Rosuvastatin calcium developed by UV-visible Spectrophotometer was linear with R^2 value 0.9994 shown in the Fig-2 & 3. The drug-excipients compatibility showed all the excipients were compatible with the drug shown in the Fig-4 & 5. In the present research work Rosuvastatin niosomes were prepared using various non-ionic surfactants (Span 60, Span 80, Tween 40 & Tween 60) along with cholesterol and soya lecithin to stabilize the formed niosomes in different proportions by the thin film hydration method by CCD shown in Fig-6. The prepared Rosuvastatin niosomes were evaluated for various parameters like particle size, Poly dispersibility index, zeta potential, entrapment efficiency and *drug content*. The RN-R2 niosomal formulation was considered as an optimised dispersion with a least size (211.6 nm) among all the formulations with good PDI (0.197) and zeta potential (-44.76 mV) shown in Fig-7 & 8. RN-R2 formulation showed good entrapment efficiency and drug release. Transmission electron microscopy analysis shown the niosomes were spherical and in nano range shown in Fig-9.

The daily dose of RS in patches selected is 20 mg and each circular patch of 2 × 2 cm^2 patch contain 20 mg of RS. The best placebo patches combinations were used for further studies, the patches were evaluated for various parameters and the best niosomal patch RN-T2 shown Weight Variation (364±21.5 mg), thickness (0.312±0.13mm), % moisture content (0.72±0.029), folding endurance (>200), drug content (100.95±1.71) and surface pH (6.73±0.22). Invitro diffusion studies of the patch shown RN-T6 released upto 24 hours with %CDR of 98.98% and the drug diffusion data is fitted into various mathematical models and RN-T6 followed zero order drug release kinetics and the diffusion mechanism was non-fickian diffusion depicted in the table-5.

4. Conclusion

Rosuvastatin calcium is a drug belonging to the Biopharmaceutics Classification System (BCS) class with low solubility. Rosuvastatin niosomes have been successfully incorporated into a transdermal patch. The patch is formulated using HPMC E5 and HPMC 15cps, which are hydrophilic polymers commonly used in transdermal drug delivery systems. The transdermal patch is designed to release a 20 mg dose of Rosuvastatin niosomes over a period of 24 hours. This sustained release profile ensures that the drug is continuously delivered to the systemic circulation, thus enhancing patient compliance, especially in the treatment of chronic hypertension.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

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