FORMULATION AND CHARACTERIZATIONS OF ROSUVASTATIN LOADED NANOSUSPENSION

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ABSTRACT

Nanosuspensions (NS) are novel means of delivering drugs in controlled manner used to enhance bioavailability and get controlled therapeutic effect. Thus, the aim of the current study was to develop and optimize the preparation and characterization methods of Nanosuspensions for poorly water-soluble drug compound Rosuvastatin Ca (ROS) using Box Behnken Design (BBD). Pre formulation studies included differential scanning Calorimetry (DSC), Infra-Red (FTIR) spectroscopy and X-Ray diffraction (XRD) analysis were carried out to check compatibility of ROS and other excipient before starting optimization modeling by Box Behnken Design. The designed fifteen formulae of Nanosuspension were prepared and monitored for different responses to determine optimum formula and its expected characterization parameters. Then testing the efficiency of optimum formula against selected responses. Rosuvastatin Ca (ROS) nano suspension formulating was prepared using thin film hydration method using Box Behnken Design modeling include three independent variables (cholesterol (X1), Soy lecithin (X2), and span 60 (X3)), The responses were coded Y1 to Y6 respectively (particle size (PS), zeta potential (ZP), entrapment efficiency percent (EE%), as well as in vitro drug release after four hours, eight hours, and twelve hours. After obtaining optimum formula it was monitored for responses Y1 to Y6. The values of studied responses were particle size (408.6 nm), zeta potential (-53 mv), entrapment efficiency (79.2 %), cumulative drug release at 4 hr (53.2 %), cumulative drug release at 8 hr (67.1 %) and cumulative drug release at 12 hr (86.8 %). The results demonstrated that BBD optimization technique succeeded in prediction of an optimized ROS NS formula which when prepared and investigated, it met the demands of the desired responses comparing with free Rosuvastatin.

Keywords: Nano Suspension (NS), Rosuvastatin Ca (ROS), Span 60, Cholesterol, Lecithin, Box Behnken Design (BBD).

Introduction

Nanosuspensions technology has been developed as an efficient strategy for efficient delivery of water insoluble drugs **(Pires et al., 2022).** This method is applied to poorly soluble drugs that are insoluble in both water and oils. It produces a physically more stable product than liposome; conventional colloidal drug carriers. The difference between Nanosuspensions and Nanoparticles is Nanoparticles are commonly polymeric colloidal carriers of drugs whereas Nanosuspensions are such hydrated vesicular systems obtained by hydration of synthetic non-ionic surfactants along with different stabilizers **(Amoabediny et al., 2018).**

Rosuvastatin Ca (ROS) is a synthetic lipid lowering agent with poor water solubility, which falls on bio pharmaceutical classification system (BCS) Class II. Since drugs under BCS Class II are not easily dissolved having low solubility and high permeability, **(Amrutha et al., 2020)**, they may not get absorbed from GIT **(Poovi et al., 2018)**.

Box and Behnken **(Beg et al., 2021)** have proposed some designs for a spherical domain whose most specific property is that each factor takes only three levels. The class of designs is based on the construction of balanced in completed block designs. The design for three variables is formed by three blocks; in each of them, two variables are combined following a 22 factorial design and the remaining third variable is maintained at level zero. Also, several center points are added.

In the current study, we used BBD to formulate NS of ROS with three independent variables to study six responses to reach final optimum formula that have best responses values as follow: the three independent variables were amount of cholesterol, lecithin, and span 60 (all in mg) were coded From X1 to X3 respectively. The six responses were particle size (PS), zeta potential (ZP), entrapment efficiency (EE%), in-vitro drug release after four hours, eight hours, and twelve hours and were coded from (Y1 toY6) respectively. After that the obtained fifteen formulae formulated by thin layer hydration method and the values of resulted six responses were added using mini tab software version 18 as the following optimizing parameters Y1 minimize, Y2 maximize, Y3 maximize, Y4 maximize, Y5 minimize (in order to control ROS in-vitro release), and Y6 maximize to obtain optimized formula (OPT formula). OPT formula was formulated and its responses were tested to compare between their theoretical values and their actual values and summarize results.

Experimental Materials

Rosuvastatin calcium kindly supplied by Multi Apex company as a gift sample (Cairo, Egypt). Cholesterol from sigma Aldrich, USA. Soy lecithin from sigma Aldrich, USA. Span 60 from sigma Aldrich, USA. Chloroform with analytical grade (obtained from Merck Millipore, Germany). Highly purified milli-Q water (resistivity of 18.2 MX cm). Acetonitrile (HPLC grade) was supplied by Wako Pure Chemical, Osaka, (Japan), methanol (analytical grade supplied by Merck Millipore, Germany). The buffer used were prepared from standard buffer tablets supplied by (Loba Chemie Pvt Ltd, India) and adjusting the pH by either 0.1 N HCL or Na OH.

Pre-formulation studies

All the preformulation studies were carried out by the powders of the active drug and its additives, while nanosuspensions were liquid form.

Differential scanning calorimetry (DSC)

Determination of the compatibility of Rosuvastatin Ca (ROS) with different excipients namely cholesterol, soy lecithin, and span 60 were investigated using Differential scanning Calorimeter (DSC), Perkin Elmer Q2000 Exton, PA. Approximately 5mg of samples were weighed and placed in the perforated aluminum sealed pans and heated at rate of 10°C/min, with indium in the reference pan in an atmosphere of nitrogen flow (50 ml/min) between 50 and 300°C so the temperature of all were heated at constant rate. The DSC studies were carried out for Rosuvastatin, each excipient and physical mixture of Rosuvastatin with the investigated excipients (1:1W/W).

Fourier-transform infrared spectroscopy (FTIR)

According to **Sarfraz et al., (2017)**, IR spectra for Rosuvastatin Calcium, Phospholipid (soy lecithin), cholesterol and Rosuvastatin-Phospholipid mixture were obtained on an FT-IR Spectrometer, Perkin Elmer, USA. In the transmission mode with the wave number region 3500 - 100 cm^{-1} IR spectra for ROS, each excipient and physical mixture of ROS were investigated in ratio of (1:1W/W).

X-ray diffraction analysis

X-Ray diffraction analysis was performed using X-Ray diffractometer to characterize the crystalline phases and detect the amorphous structure in the samples **(Ippili et al., 2018)**. The crystalline state of Rosuvastatin Calcium was evaluated with X-ray powder diffraction. The X-ray generator was operated at 40 KV tube voltages and 40 mA of tube current, using the Ka lines of copper as the radiation source. The scanning angle ranged from 1 to 600 of 2θ in step scan mode

Analytical method development of Rosuvastatin calcium (ROS Ca)

UV scanning of ROS Ca

UV spectrum of Rosuvastatin was carried out in methanol solution as described by **Gupta et al., (2009)** with simple modification. In brief, ROS (100 mg) was accurately weighed and dissolved in 100 ml of methanol to form a stock solution (1000 µg/ml). The stock solution was further diluted suitably with methanol to get a standard solution of concentration 100 µg/ml. Standard was scanned at range of 100 – 4000 nm, to the wave length with maximum absorption. The previous steps were repeated using phosphate buffer solution (pH 6.8) and scanned at range of 100 – 4000 nm to obtain wave length with maximum absorbance.

Construction of calibration curve of ROS Ca

UV Calibration Curve used in in-vitro release study

In brief, ROS (100 mg) was accurately weighed and dissolved in 100 mL of phosphate buffer (pH 6.8) to form a stock solution (1000 µg/ml). The stock solution was further diluted suitably with buffer to get a standard solution of concentration 100 µg/ml Then working standard were scanned at range 100 – 4000 nm, the wave length with maximum absorption was determined

Aliquots of 0.2, 0.4, 0.6, 0.8, 1.0, 1.2, 1.4, 1.6 and1.8 ml of working standard solution corresponding to 2-18 µg of ROS Ca were taken in a series of 10 ml volumetric flask and volume made up with buffer.

The absorbance measurements of these solutions were carried out spectrophotometrically using Ultraviolet spectrophotometer, UV- 1800, Shimadzu, Kyoto, Japan against buffer as blank at the calculated maximum absorbance of ROS Ca. A calibration curve of Rosuvastatin was plotted. The linear correlation was obtained between absorbance and concentration of drug.

HPLC Calibration Curve used calculate entrapment efficiency percentage

Determination of ROS was performed according to the following method. The high-performance liquid chromatography (HPLC) system used was HPLC thermo fisher, USA. equipped with a UV / VIS detector (thermo fisher, USA),

The mobile phase (acetonitrile / water, and ortho phosphoric acid (40:60:1, v/ v / v) was flowed over a reversed- phase C18 column (Hypersil BDS, 4.6 × 150 mm, 5 µm particle size). The effluent was monitored at a flow rate of 2.0 ml / min at room temperature. Aliquot (100 µL) of the samples was injected and the drug was quantified by its UV absorbance at λ_{max} 242 nm and using a calibration curve (R^2: 0.999). Retention time was 4.5 minutes, with total run time 6 minutes.

Box Behnken Design (BBD) modeling

Box Behnken Design used minimum and maximum quantities of three variables (X1 to X3) in BBD modeling at mini tab software version 18 to obtain fifteen formulae. The obtained formulae were prepared by thin layer hydration method to obtain optimum formula.

Preparation of Nanosuspension

NS particles were prepared by thin film hydration method as reported previously with some modifications as described by **Weng et al., (2019)** as follow: Weighed quantity of drug, cholesterol, surfactant and Soy lecithin were dissolved in chloroform and bath sonicated for 30 minutes then taken in a round bottom flask. The flask was rotated by using rotary flash evaporator at 100 rpm for 20 minutes in a thermostatically controlled water bath at 60°C± 2°C.The flask was rotated under

reduced pressure (10-15mm mercury) until all the organic phase evaporated and a slimy layer was deposited on the wall of a round bottom flask. Then, phosphate buffer saline pH 6.8 (10ml) was used to hydrate the lipid film and the flask was rotated at the same speed and temperature but without vacuum for another 30 minutes for lipid film removal and dispersion.

Characterization of nanosuspension

Physicochemical properties of nanosuspension

The average PS and ZP were measured for the ROS NS by using dynamic light scattering zeta sizer Malvern® Zeta sizer Nano Zs 90 Malvern® Instruments Limited, Worcestershire, (UK). by dynamic light scattering and Electrophoretic light scattering principle. Each sample was diluted with HEPES (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid buffer (1:100), filtered through 0.22 µm filter and gently agitated prior to testing using a 90° scattering angle at 25°C.

Entrapment efficiency (EE %)

Entrapment efficiency percent (EE%) of Nanosuspension formulations were calculated by extracting the Nanosuspension as follow: centrifuge 1 ml of each formula at 9000 rpm and 4^{o} C for 30 minutes. Then remove the supernatant by drawing out using pipette. Extract residue and add 5ml Acetonitrile and bath sonicated for 20 minutes to let drug free from its vesicles. Then inject 100 µl to HPLC after filtration using syringe filter 0.22 micron. The encapsulation efficiency was calculated as by the following:

$$EE\ (\%) = \frac{\text{Encapsulated amount of ROS in nanosuspension}}{\text{Total amount added}} \text{ X100}$$

Transmission electron microscopy

According to **Kassem et al., (2017)** The morphology of individual Nanosuspension was observed by transmission electron microscope (TEM) JOEL transmission electron microscope (JTEM), MODEL 1010 (JAPAN) with an accelerating voltage of 120 kV. A drop of diluted Nanosuspension of the optimized formula in distilled water (DW) was placed on to carbon-coated copper grids followed by negative staining using phosphotungstic acid (1.5%) and the grid was air dried at room temperature before loading into the microscope.

In vitro release studies

The in-vitro release of Rosuvastatin from nanosuspension formulae was carried out according to the procedure described by USP with slight modification **(USP 44 – NF 39, 2021)**. Dissolution studies were performed for the pure drug, fifteen formulae of ROS NS and for the optimized formulation using dissolution apparatus, six-spindle dissolution tester, Coply, type PTWII, (India) using paddle method at rotation speed of

50 rpm. Dissolution was carried out in phosphate buffer with pH 6.8 as a dissolution medium. The volume and temperature of the dissolution medium were 500 ml and 37.0 ^{O} C ± 0.5^{O} C. Amount equivalent to 5 mg of Rosuvastatin formulae was placed inside dialysis bag with molecular weight cut off 6-16 kilo Dalton, USA, and tied with silk string from both sides then immersed in dissolution vessel that contained 500 ml of phosphate buffer pH 6.8 as a dissolution medium.

Samples of 5 ml were withdrawn and replenished by an equal volume of pre-warmed fresh dissolution medium at predetermined time interval (1h, 2h, 3h, 4h, 6h, 8h, 12h, 14h, 16h, 18h and 24h). All samples were filtered through 0.250 µm syringe filter, the amount of dissolved Rosuvastatin was determined spectrophotometrically at 242 nm by using Ultraviolet spectrophotometer, UV- 1800, Shimadzu, Kyoto, Japan. The displayed results are the mean and ± SD. The data obtained were measured until 24 hours, but the in-vitro release level off after 12 hours.

Results and Discussion

Calibration curves of ROS

Figure (1, A) showed the standard calibration curve of ROS in mobile phase in HPLC method. The retention times of ROS in mobile phase was 3.7 min. The standard curve of ROS by HPLC method was constructed at 242 nm. An excellent linearity was obtained for ROS of concentration in range of 100 - 600 µg / ml with a good determination coefficient = 0.997. The equation obtained of the standard curve was y = 132.43x + 2208.4. while figure (1, B) showed the standard calibration curve of ROS in phosphate buffer that used in in-vitro release study

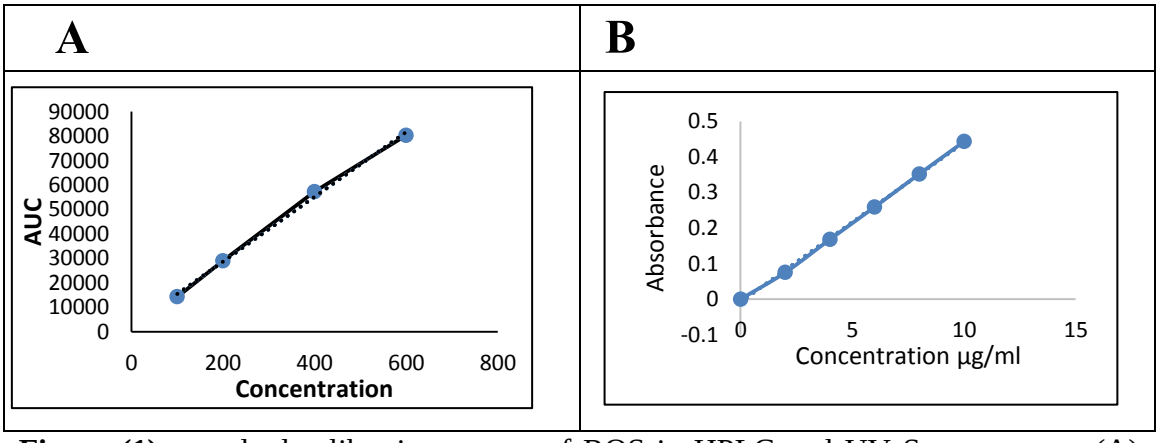

Figure (1): standard calibration curves of ROS in HPLC and UV Spectroscopy. (A) The HPLC standard calibration curve of ROS. (B) The standard curve of ROS at 242 nm by UV spectrophotometry method

Pre-Formulation study Results

Differential scanning Calorimetry (DSC):

Figure (2) showed DSC thermogram of physical mixture of ROS with other excipients. It was found that there was characteristic peak for Rosuvastatin at 146.7^{O}C, a characteristic peak of cholesterol at 149.67^{O}C and a characteristic peak of Soy lecithin at 240^{O}C and 262^{O}C. These results showed minimal change in the melting points of all components. Presence of all peaks indicated that all ingredients were compatible with drug in this study.

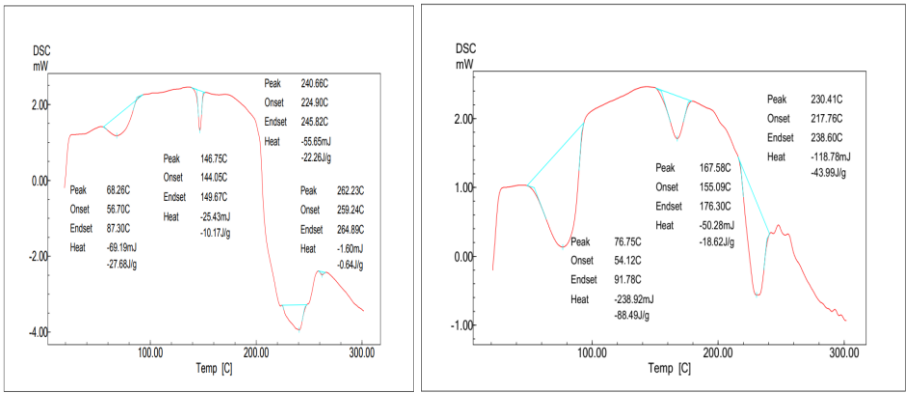

Figure (2): DSC thermogram of physical mixture and free drug

Fourier-transform infrared spectroscopy (FTIR):

Figure (3) showed that IR spectra of physical mixture and free drug indicated all characteristic peaks belonging to major functional groups which are similar to standard peaks as shown in below, Presence of all peaks in physical mixture indicates that no interference occurs between drug and excipients.

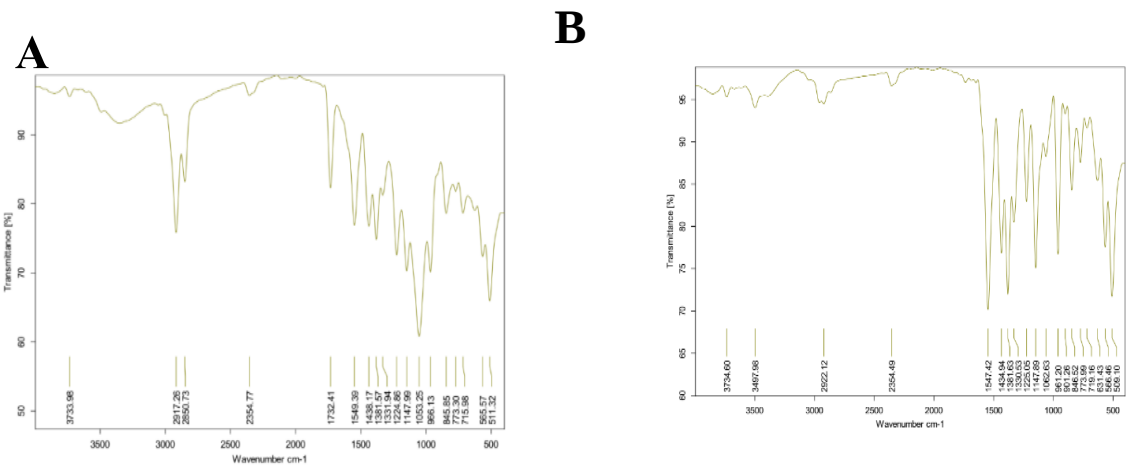

Figure (3): (A) FTIR spectra of physical mixture, (B) FTIR spectrum of free drug

X-ray diffraction analysis:

Figure (4) showed the X-ray Diffractogram of Rosuvastatin, cholesterol, soy lecithin and physical mixture the diffractogram indicated that there was no interference between drug and other excipients.

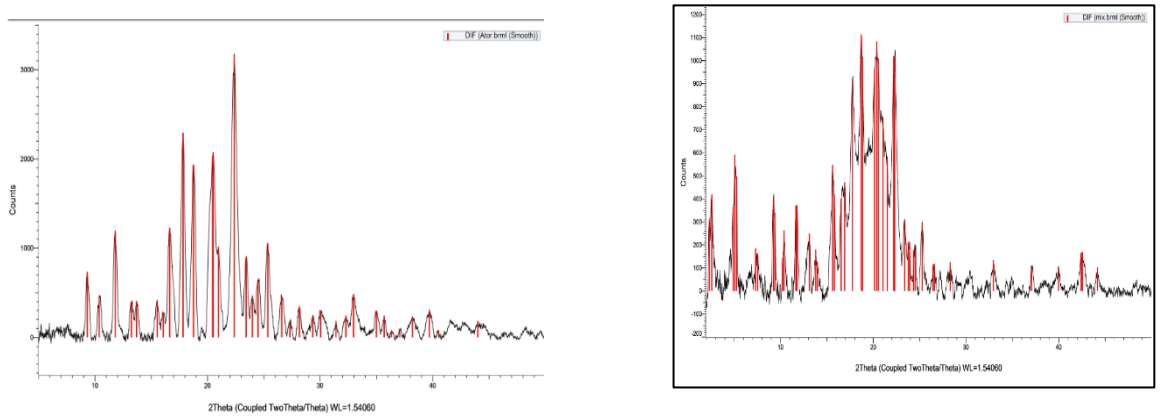

Figure (4): X-ray diffractogram of free drug and physical mixture

Box Behnken Design optimization model:

Preliminary screening was done to select optimum concentration of X1, X2, and X3 and to determine minimum and maximum values of X1, X2, and X3 and the results tabulated in **table (1). Table (2)** summarize fifteen formulae that produced from BBD using Mini tab version 18.

Table (1): Box-Behnken Design factors and responses

	Factors	**Code**	**Minimum**	**Medium**	**maximum**
Variables	Cholesterol (mg)	X1	50	100	150
	Lecithin (mg)	X2	50	100	150
	Span 60 (mg)	X3	50	100	150
Responses	Particle size	Y1			
	Zeta potential	Y2			
	Entrapment efficiency	Y3			
	Release after 4 hours	Y4			
	Release after 8 hours	Y5			
	Release after 12 hours	Y6			

Table (2): Box-Behnken Design summary

No	ROS (mg)	X1 (mg)	X2 (mg)	X3 (mg)
F1	10	100	150	50
F2	10	50	50	100
F3	10	150	100	150
F4	10	150	100	50
F5	10	100	50	150
F6	10	100	100	100
F7	10	100	100	100
F8	10	100	100	100
F9	10	150	150	100
F10	10	50	100	50
F11	10	50	100	150
F12	10	150	50	100
F13	10	50	150	100
F14	10	100	150	150
F15	10	100	50	50

Characterization of ROS NS:

the values of corresponding responses (Y1 – Y6) for the fifteen prepared NS formulae were tabulated in table (3)

Table (3): Experimental run and their observed responses

No	X1	X2	X3	Y1	Y2	Y3	Y4	Y5	Y6
F1	100	150	50	410.5 ± 2.1	-51.2 ± 1.5	75.6 ± 2.1	41.89 ± 1.3	60.25 ± 1.4	82.61 ± 2.1
F2	50	50	100	427.6 ± 2.8	-54.8 ± 1.3	70.6 ± 2.1	50.2 ± 1.5	63.4 ± 2.6	85.4 ± 2.3
F3	150	100	150	480.6 ± 1.9	-57.6 ± 2.1	88.6 ± 2.1	51.27 ± 1.7	75.46 ± 1.1	91.47 ± 2.3
F4	150	100	50	430.2 ± 1.8	-52.7 ± 1.4	78.6 ± 1.3	38.61 ± 1.6	55.72 ± 1.4	76.7 ± 2.7
F5	100	50	150	430.5 ± 3.1	-55.3 ± 2.1	80.4 ± 2.6	60.3 ± 1.7	72.5 ± 2.4	91.37 ± 2.0
F6	100	100	100	404.4 ± 3.7	-55.2 ± 1.9	78.6 ± 2.1	55.4 ± 2.6	67.4 ± 1.6	86.5 ± 2.1
F7	100	100	100	402.3 ± 3.5	-57.2 ± 1.3	77.6 ± 2.4	56.4 ± 2.1	68.4 ± 1.3	87.5 ± 2.4
F8	100	100	100	406.4 ± 2.7	-59.2 ± 1.7	78.9 ± 2.6	54.4 ± 2.3	69.1± 1.7	86.1 ± 2.6
F9	150	150	100	405.4 ± 2.3	-49.2 ± 2.1	84.5 ± 1.8	55.3 ± 1.7	68.4 ± 1.4	89.9 ± 3.4
F10	50	100	50	411.4 ± 2.1	-57.2 ± 2.1	70.6 ± 1.1	57.6 ± 1.3	76.8 ± 2.4	89.7± 3.9
F11	50	100	150	470.6 ± 3.4	-48.2 ± 1.6	78.6 ± 1.5	65.4 ± 2.3	77.4± 2.8	93.4 ± 2.9
F12	150	50	100	380.5 ± 2.9	-48.1 ± 1.7	77.4 ± 2.3	40.3 ± 2.4	61.6 ± 1.7	76.4 ± 2.7
F13	50	150	100	490.4 ± 2.4	53.5 ± 1.5	78.2 ± 2.4	60.4 ± 1.7	75.8 ± 1.6	90.1 ± 2.4
F14	100	150	150	448.3 ± 3.3	-58.4 ± 2.1	87.7 ± 2.8	61.24 ± 1.1	89.38 ± 2.2	98.34 ± 3.9
F15	100	50	50	370.4 ± 2.6	-53.5 ± 2.9	69.4 ± 2.6	41.6 ± 2.9	57.6 ± 2.6	70.8 ± 3.3

Measurement of Particle size (Y1):

So, the rank order of the determined particle sizes in these results can be arranged, in an ascending manner, as follows: F 15 < F 12 < F 7 < F 6 < F9 < F 8 < F 1 < F 10 < F 2 < F 4 < F 5 < F 14 < F 11 < F 3 and < F 13.

Measurement of Zeta Potential (Y2):

The Rosuvastatin Nanosuspension in this study displayed a relatively high negative zeta potential. This high value of ZP ensures that the ROS NS will have a very good stability and tolerance against aggregation.

Drug Entrapment efficiency percent (Y3):

From the obtained results, it was found that EE was ranged from 67.4 ± 1.6 % (F15) and 88.6 ± 2.7 % (F3).

So, the rank order of the determined EE % can be arranged, in a descending manner, as follows: F 3 > F 14 > F 9 > F 5 > F 4 > F 11 > F 13 > F 6 = F 7 = F 8 > F 12 > F 1 > F 2 = F 10 and > F 15.

In-vitro drug release (Y4 toY6):

The all over the in-vitro release of Rosuvastatin formulae have marked increase with controlled pattern when compared to pure Rosuvastatin itself. figure (5) showed that in-vitro release data of OPT formula and free drug

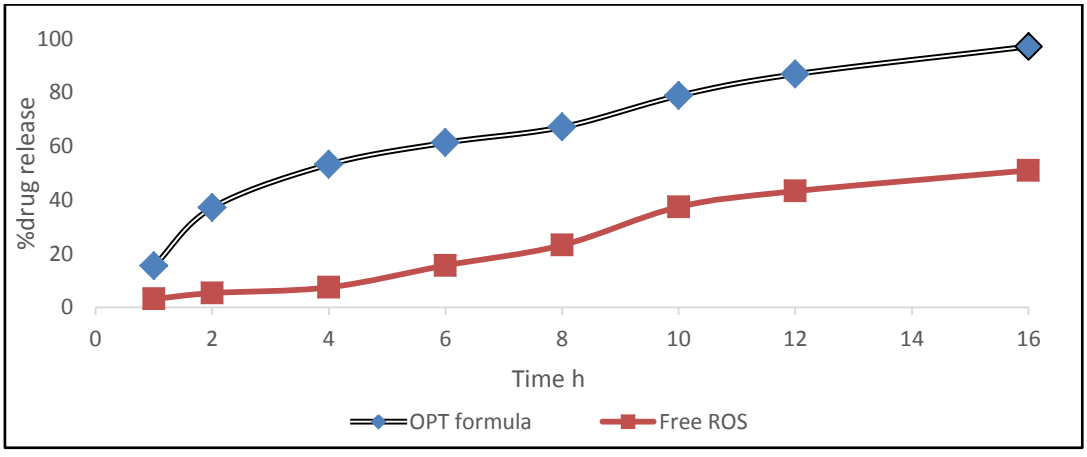

Figure (5): Invitro release data of OPT formula and free drug

Kinetics of in- vitro release

Table (4) showed the R values of in-vitro drug release that obtained for fifteen formulae and free drug for different kinetic models.

Table (4): (r) values for different kinetic models of in-vitro drug release for fifteen formulae and free drug

Formula	zero order	First order	**Higuchi**
F1	0.9877	-0.7692	**0.9963**
F2	0.9759	-0.7967	**0.9936**
F3	0.9665	-0.8333	**0.9951**
F4	0.9871	-0.8957	**0.9943**
F5	0.9518	-0.8033	**0.9877**
F6	0.9589	-0.7832	**0.9896**
F7	0.9589	-0.7832	**0.9896**
F8	0.9589	-0.7832	**0.9896**
F9	0.9618	-0.7918	**0.9915**
F10	0.9610	-0.7912	**0.9930**
F11	0.9377	-0.8138	**0.9816**
F12	0.9925	-0.7509	**0.9832**
F13	0.9611	-0.7961	**0.9922**
F14	0.9070	-0.8893	**0.9661**
F15	0.9840	-0.9054	**0.9879**
Pure ROS	**0.9836**	-0.9820	0.9612

The model that gives higher (r) value was considered as the best fit model. The (r) values were found to be higher in the Higuchi model which described that the release of ROS NS carried out by diffusion mechanism from the prepared formulae this agreed with **Dash et al (2010)**.

Optimization parameters and response optimizer

The targeted response parameters were smaller PS, higher ZP, higher EE%, higher cumulative drug release (CDR%) after 4 hours, lower CDR5 after 8 hours and higher CDR% after 12 hours. The responses were statistically analyzed using Mini Tab software. The individual parameters were evaluated using the F test and quadratic models of the form

$$\mathbf{Y = \beta0 + \beta1\ X\ 1 + \beta2\ X\ 2 + \beta3\ X\ 3 + \beta4\ X\ 1\ X\ 2 + \beta4\ X^2 1 + \beta5\ X^2 2 + \beta6\ X^2\ 3 + \beta7\ X1\ X2 + \beta8\ X1\ X3 + \beta8\ X2\ X3}$$

Where, Y is the level of the measured response; β0 is the intercept, β1 to β8 are the regression coefficients. X1, X2 and X3 stand for the main effects; X1 X2, X1 X3 and X2 X3 is the interaction between the main effects; $X1^2$, $X2^2$ and $X3^2$ are the quadratic terms of the independent variables that were used to simulate the curvature of the designed sample space.

A backward elimination procedure was adopted to fit the data into different predictor equations. The quadratic models generated by regression analysis were used to construct the 3-dimensional graphs in which response parameter Y was represented by a

curvature surface as a function of X. The effect of the independent variables on each response parameters was visualized from the contour plots.

An optimized formulation was developed by setting constraints on the dependent and independent variables. The optimum formula was evaluated for the responses and the experimental values obtained were compared with those predicted by the mathematical models, the factorial design analysis approach is a helpful tool during formulation optimization as it allows elucidating the relationship between the dependent and independent variables during the optimization process by providing the main effects.

Statistical analysis of optimization parameters

Effect of X1, X2 and X3 on PS

The PS of the tested ROS NS ranged from 370.4 to 490.4 nm and the mathematical polynomial model equation for the mean particle size (Y1) was

$$\mathbf{Y1 = 457.2 - 2.461\ X_1 + 0.364\ X_2 + 0.519\ X_3 + 0.01101\ X_1^2}$$

In which the negative sign of X1 (cholesterol) indicates antagonistic effect, if the cholesterol increases the particle size decrease, and the positive sign of X2 (lecithin) indicates synergistic effect, by increasing lecithin particle size increase as it was illustrated in figure (6). The improvement of PS by increasing cholesterol and decreasing lecithin and span 60 due to the fact that the addition of cholesterol can enhance the hydrophobicity, leading to a decrease in the surface free energy and therefore decrease of particle size. Lecithin and span 60 have synergistic effect on particle size due to the fact that Lecithin increases drug adherence to the surface of the nanoparticles and the increase in the size of the nanoparticles. The obtained results for measuring the PS of ROS NS were in good agreement with the published papers from the work of **Yousfan et al., (2020)**.

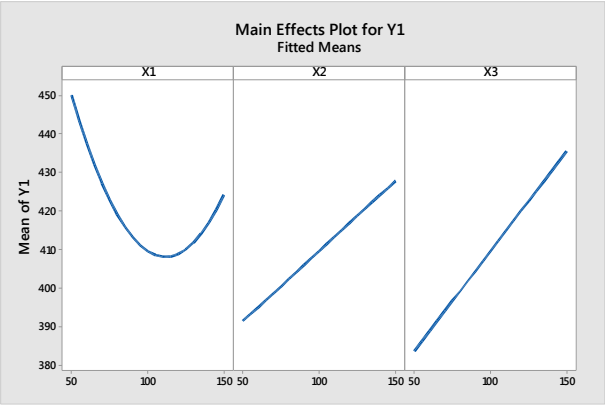

Figure (6): Main effect plot of X1 and X2 and X3 on PS

Effect of X1, X2 and X3 on ZP:

The resulted values of ZP ranged from - 48.1 mV to - 58.3 mV and the mathematical polynomial model equation for the mean ZP (Y2) was

$$\mathbf{Y2 = 57.71 + 0.090\ X_1 - 0.1268\ X_3 - 0.001221\ X_1^2 + 0.001390\ X_1X_3}$$

In which positive sign coefficients of X1(β1) indicated a synergistic effect as the cholesterol increased, the ZP significantly increased. And negative sign of β3 span 60 indicated antagonistic effect. This main effect was illustrated in figure (7). The improvement in the ZP with increasing X1 and X3 factors was due to the fact that the ZP increased with increasing both cholesterol and Span 60 which act as steric barrier through adsorption on the droplet surface which allowed for preventing close contact of the droplets and hence prevented agglomeration of the drug particles. The obtained results were in agreement with **Chaudhari et al., (2020).**

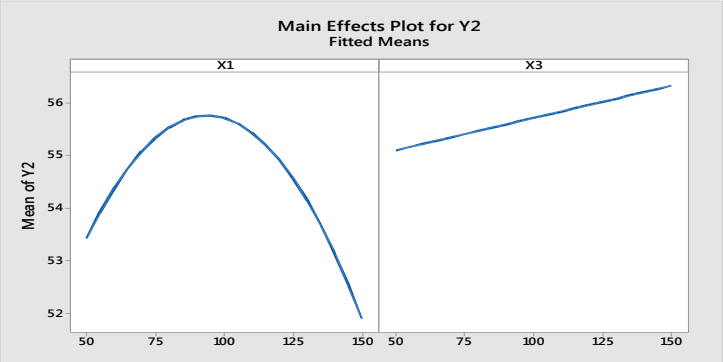

Figure (7): Main effect plot of X1 and X3 on ZP

Effect of X1, X2 and X3 on EE%:

The EE values was ranged from (67.4 to 88.6 %). The mathematical polynomial model equation for the mean particle size (Y3) was

$$\mathbf{Y3 = 56.72 + 0.07775\ X_1 + 0.07050\ X_2 + 0.0192\ X_3 + 0.000418\ X_3^2}$$

In which the positive sign for regression coefficients of X1, X2 and X3 (β1, β2 and β3) indicated a synergistic effect as cholesterol and lecithin and span 60 concentrations increased, the EE significantly increased, as it was illustrated in figure (8). The improvement of EE with increasing X1 was due to the fact that cholesterol improves the rigidity of the vesicular membrane and positively affects the permeability of system with high drug encapsulation efficacy. Other factors X2 and X3 also increase EE due to amphiphilic polymers Soy lecithin and span 60 which acts as surfactant to reduce the interfacial tension between the polymer and aqueous phase and so producing highest EE. The results of EE% coincided with the work done by **Yang et al. (2015).**

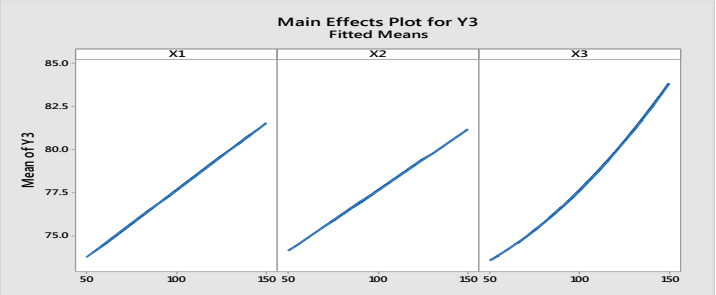

Figure (8): Main effect plot of X1 and X2 and X3 on EE

Effect of X1, X2 and X3 on in-vitro drug release:

The mathematical polynomial model equations for the in-vitro drug release after 4 h (Y5), 8 h (Y5), and 12 hours (Y3) were:

$$Y4 = 80.63 - 0.1710\ X_1 + 0.0929\ X_2 + 0.0267\ X_3 + 0.001107\ X_1X_3$$

$$Y5 = 99.2 - 0.2969\ X_1 - 0.0205\ X_2 + 0.516\ X_3 + 0.001718\ X_3^2 + 0.001914\ X_1X_3 + 0.001423\ X_2X_3$$

$$Y6 = 80.63 - 0.1710\ X_1 + 0.0929\ X_2 + 0.0267\ X_3 + 0.001107\ X_1X_3$$

In which β1 is a negative sign which indicated antagonistic effect on drug release, while β2 and β3 are positive show synergistic effect, this effect was illustrated in figure (9). The antagonistic effect of cholesterol on % CDR due to the fact that inclusion of cholesterol in the formula decreases the permeability of the vesicular membrane to various solutes, hence controlled release of the drug. The synergistic effect of X2 and X3 lecithin and span 60 may be due to the fact thatX2 and X3 are surfactant that both factors in decreasing the particle size of the drug to be in the nonorange which aid in increasing the wettability and solubility of poorly water-soluble drug, hence increasing the %CDR. The results of the in-vitro release after 4 hours in this study coincided with the work done by **Premathilaka et al., (2022)** and **Vlasova et al., (2019).**

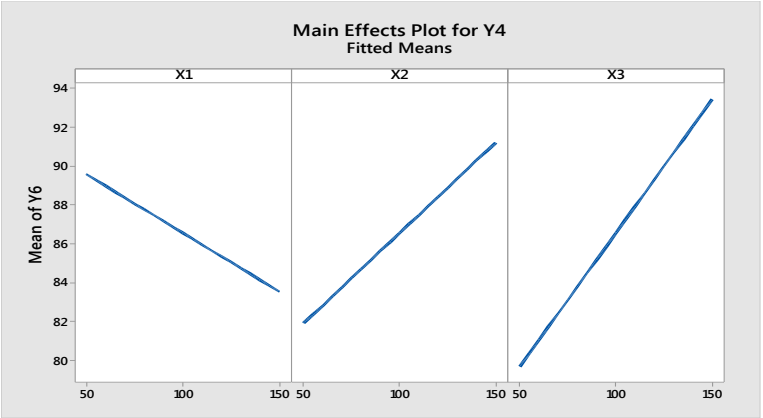

Figure (9): Main effects plot of X1 and X2 and X3 on % CDR

Multiple response optimization

The optimum formula of ROS system was selected based on the criteria of attaining the maximum cumulative percent drug release at 4 hours and at 12 hours, Zeta potential, EE% and minimizing the particle size and % CDR at 8 hours by applying Point prediction method (multiple response optimization) of the Mini Tab software. The formula composition with cholesterol (115.6 mg), Soy lecithin (53.1 mg), span 60 (150 mg) and Rosuvastatin Ca (10 mg) was prepared and characterized

Characterization of OPT formula:

Particle size and zeta potential

Particle size distribution of the optimized formula was shown in figure (10A) where the average particle size of optimized batch (OPT-ROS) is 408.6 nm while zeta potential was found to be -53 mV as shown in Figure (10B).

A **B**

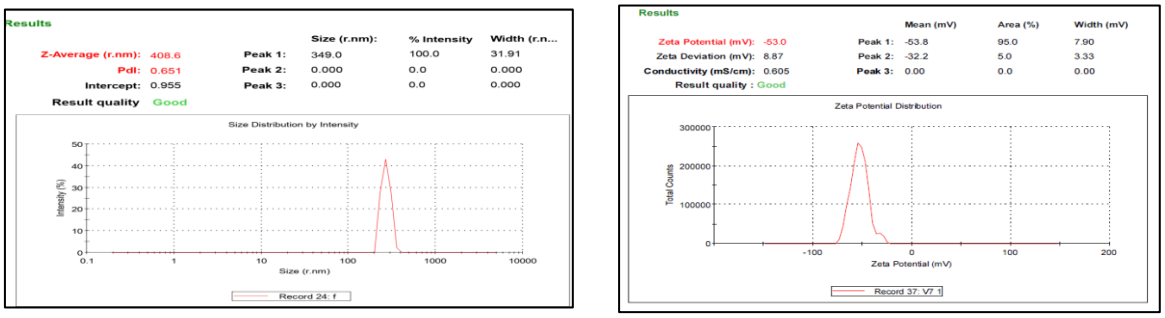

Figure (10): Characterization of OPT formula. (A) particle size of the optimized formula. (B) Zeta potential of the optimized formula

Transmission electronic microscope (TEM)

Figure (11) showed TEM Image of the optimized ROS. images revealed that there is no aggregation of nanosuspension. It was also observed that NS are not of uniform size and approximately of oval Shape.

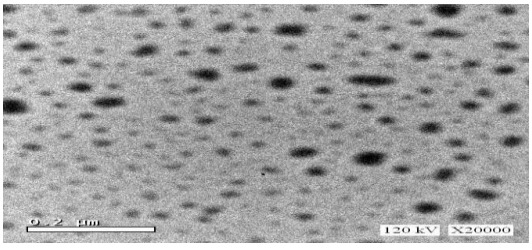

Figure (11): TEM Image of the optimized ROS

Statistical analysis of the OPT formula results:

Resulted values of all responses Y1 – Y6 for the optimum formula were compared with that expected from the optimization design. The statistical analysis of OPT formula values revealed that there was no significant difference between predicted and observed values. The results were shown in table (5) that there was no significant difference between Predicted and observed values.

Table (5): Predicted and Observed value of the dependent responses of optimized ROS

DEPENDENT RESPONSE	OBSERVED	PREDICTED
Y1: PS	408.6	417
Y2: ZP	-53	-56.88
Y3: %EE	79.2	81.7
Y4: %CDR AT 4 hr	53.2	55.22
Y5: %CDR AT 8 hr	67.1	69.48
Y6: %CDR AT 12 hr	86.8	89

CONCLUSION

The obtained results of this work revealed that nanosuspension was a suitable novel method delivering ROS as a poorly water-soluble drug. It was also revealed that the success of box Behnken design as optimization model in prediction of an optimum formula of ROS loaded nanosuspension. This formula fulfilled the fundamentals demands of USP for nanosized particle size (408.6 nm), zeta potential (-53 mv), entrapment efficiency (79.2 %), cumulative drug release at 4 hr (53.2 %), cumulative drug release at 8 hr (67.1 %) and cumulative drug release at 12 hr (86.8 %).

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صياغة وتوصيف المعلقات النانومترية لعقار الرسوفاستاتين

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تعتبر المعلقات النانومتريه هي وسيلة جديدة للتحكم في توصيل الدواء للانسجة ، لتعزيز التوافر البيولوجي والحصول على تأثير علاجي منتظم. وبالتالي ، كان الهدف من الدراسة الحالية هو تطوير استمثال طرق التحضير والتوصيف والفاعلية لدواء ضعيف الذوبان في الماء (رسوفاستاتين كالسيوم) باستخدام نموذج بوكس بينكن (BBD) ، وتشمل دراسات الصياغة المسبقة قياس المسح التفاضلي (DSC) ،و إجراء التحليل الطيفي بالأشعة تحت الحمراء (FTIR) ، وتحليل حيود الأشعة السينية (XRD) للتحقق من توافق الدواء والسواغات الأخرى قبل البدء في الاستمثال بواسطة تصميم بوكس بينكن ، وتم الحصول على خمسة عشر صيغة من المعلقات النانويه و اختيار الاستجابات المختلفة لتحديد الصيغة المثلى و قيم التوصيف المتوقعة لها ، ودراسة كفاءة الصيغة المثلى في الاستجابات المختارة. تم تحضير معلق روسوفاستاتين النانوي باستخدام طريقة التبخير ثم الترطيب للطبقة الرقيقة باستخدام نموذج بوكس بينكن على ثلاثة متغيرات مستقلة (الكوليسترول (X1) ، والليسيثين (X2) ، و سبان ٦٠ (X3)) ، ثم حدد الاستجابات المختارة التالية المكودة من Y1 إلى Y6 على الترتيب (حجم الجسيم (PS) ، جهد زيتا (ZP) ، كفاءة الحصر (EE) ، وكذلك انطلاق الدواء بعد أربع ساعات وثماني ساعات واثنتي عشرة ساعة. بعد الحصول على الصيغة المثلى ، تم اختبار الاستجابات من Y1 إلى Y6. و قد أظهرت النتائج أن تقنية تحسين BBD نجحت في التنبؤ بصيغة ROS NS مثلى والتي عند تحضيرها وفحصها قد استوفت متطلبات الاستجابات المرغوبة مقارنة مع الدواء العادي بدون تقنية النانو.

الكلمات المفتاحية : المعلقات النانومتريه ، رسوفاستاتين كالسيوم ، الكوليسترول ، الليسيثين ، و سبان ٦٠ ، نموذج بوكس بينكن