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Enhancement of the Solubility of Rosuvastatin Calcium by Nanovesicular Formulation: A Systematic Study Based on Quality by Design Approach †

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Abstract: Rosuvastatin calcium (Rsv) is an effective statin, with a potent antihyperlipidemic effect. However, it suffers poor bioavailability owing to its poor solubility. Thus; encapsulating Rsv into a nanovesicular structure could overcome this problem. The aim of this work is to investigate the potential of solid lipid nanoparticles (SLN) and nanostructured lipid carriers (NLC) in enhancing the solubility of Rsv, using Quality by Design (QbD) concept. A complete risk assessment study has been conducted, where the critical process parameters (CPP), material attributes (MA), and critical quality attributes have been identified using the ishikawa diagrams. Selected CPP/MA were screened and further upgraded to a 2^4 full factorial design to develop the design space with the optimized formula. The screened CPP/MA were tested on each of the particle size, polydispersity index (PDI), zeta potential (ζ-pot) and the entrapment efficiency (EE%). A comprehensive approach for Rsv nanovesicular carriers has been conducted, where the NLC showed better results than the SLN. The optimized formula was prepared with 3% total lipid content, 0.154% surfactant, and 9.4 mg drug. The optimized formula had a particle size of 310.5 nm, with 0.243 PDI, a ζ-pot of −24.7 mV and EE% of 93.87%, and showed a sustained release of the drug up to 72 h. It successfully lowered each of the total cholesterol, low density lipoprotein, and triglycerides and elevated the level of the high density lipoprotein of the rats, with better results as compared to the standard drug. Thus, a complete QbD study was conducted to explore experimental regions for many successful nanovesicular carriers for the enhancement of the solubility of poorly soluble drugs.

Keywords: quality by design; solid lipid nanoparticles; nanostructured lipid carriers; antihyperlipedemia

1. Introduction

Quality by Design (QbD) is a systematic science and risk-based approach that plays a great role in product and process understanding in order to achieve a safe product. The use of QbD in pharmaceutical formulation assures the quality of pharmaceutical product by scientific development and risk management tools, producing a high quality product in the most efficient manner [1]. Rosuvastatin calcium (Rsv) is one of the most effective statins, with the potential of reducing low-density lipoprotein (LDL), triglycerides (TG) and increasing high-density lipoprotein (HDL). However, Rsv suffers from poor solubility and extensive firs pass effect resulting in its poor bioavailability [2]. Solid lipid nanoparticles (SLN) and nanostructured lipid carriers (NLC) are vesicular nanoparticles made from physiologically accepted and biodegradable lipid fractions [3].

The objective of the current study is the application of QbD approach in the optimization and formulation of Rsv; in an attempt to improve its solubility.

2. Experiments

2.1. Materials

Rosuvastatin calcium, A32700, was as a generous gift from Global Napi Pharmaceutical Company (Cairo, Egypt), Precirol® ATO 5and Compritol® 888 ATO were received as gifts from GatteFosse (Lyon, France). Tween® 20 was purchased from Sigma (St. Louis, MO, USA) while Tween® 80 from Scharlau (Barcelona, Spain). Stearic acid from Piochem, (Giza, Egypt), Oleic acid from Oxford Labchem, (Maharashtra, India) and Castor oil from UCCMA, (Cairo, Egypt). Poloxamer® 188 from Caisson smithfield, (UT, USA). All other chemicals and reagents were of analytical grade. Fructose for the *in-vivo* study was obtained from UNIPHARMA Co., (El-Obour City, Cairo, Egypt), while sheep tail fat and hydrogenated oil were from commercial sources. Sodium carboxymethyl cellulose was obtained from Chemajet Pharmaceutical Industries, (Cairo, Egypt).

2.2. Methods

2.2.1. Preparation of Nanovesicular Carrier

SLN and NLC were prepared by emulsification-ultrasonication method as reported by Das et al. [4].

2.2.2. Characterization of Nanoparticles

Particle size (PS), polydispersity index (PDI) and zeta potential (ζ-pot) measurement were performed using dynamic light scattering using zetasizer after suitable dilutions [5].The entrapment efficiency (EE%) was measured indirectly by analyzing the free drug in the supernatant after centrifugation of the dispersion [5].

2.2.3. Quality by Design Paradigm

The quality target product profile (QTPP) of the current study is to enhance the solubility of Rsv. The average PS, PDI, ζ-pot, EE% and drug release profile were taken as the influential critical quality attributes (CQA) for the current study [6].

*Risk analysis:*Identification of the failure modes was performed using Ishikawa diagrams, to figure out the critical process parameters (CPP) and material attributes (MA) affecting the QTPP [7].

Screening of different solid lipids, liquid lipids and surfactants for nanovesicular formulation: Saturated solubility of the drug in different liquid lipids (oleic acid and castor oil) and SAA (Tween® 20, Tween® 80 and Poloxamer® 188) was measeured [8]. Compritol® 888 ATO, Precirol® ATO 5, and Stearic acid were tested for their ability to solubilize Rsv [9].

Optimization of Rsv-loaded SLN/NLC with the selected variables: A 2^4 full factorial design was used for the optimization stepas shown in Table 1.

Table 1. The studied CPP/MA, their levels, and the composition of the 16 formulae as obtained from the factorial design, with the results of CQA.

Factors					**Low Level (−1)**		**High Level (+1)**	
X_1 Lipid (%)					1		3	
X_2 SAA (%)					0.1		0.3	
X_3 Solid lipid: Liquid lipid					7:3		10:0	
X_4 Drug amount (mg)					5		10	
Code	**X_1**	**X_2**	**X_3**	**X_4**	**Y_1 = PS (nm)**	**$Y2_3$ = PDI**	**Y_3 = ζ-pot (mV)**	**Y_4 = EE (%)**
F1	−1	−1	1	−1	279.2 ± 1.56	0.452 ± 0.05	−14.3 ± 2.07	45.43 ± 5.98

F2	1	−1	−1	−1	232.8 ± 3.57	0.175 ± 0.09	−19.2 ± 1.87	76.71 ± 8.83
F3	1	1	1	−1	308.9 ± 3.54	0.589 ± 0.13	−14.1 ± 4.08	81.37 ± 3.78
F4	−1	1	1	−1	400.4 ± 1.65	0.643 ± 0.08	−12.9 ± 2.98	85.57 ± 2.98
F5	1	−1	1	1	400.3 ± 2.56	0.384 ± 0.04	−14.7 ± 0.98	67.12 ± 4.14
F6	1	−1	−1	1	300.0 ± 2.73	0.228 ± 0.05	−21.4 ± 3.09	94.40 ± 9.06
F7	1	1	−1	1	255.0 ± 2.90	0.292 ± 0.09	−16.6 ± 4.09	89.20 ± 3.87
F8	−1	1	−1	1	256.5 ± 1.65	0.400 ± 0.04	−16.3 ± 1.87	77.39 ± 5.76
F9	−1	−1	−1	1	806.1 ± 2.63	0.538 ± 0.06	−18.1 ± 3.04	78.67 ± 4.31
F10	1	1	1	1	313.1 ± 0.95	0.270 ± 0.08	−13.5 ± 1.98	82.96 ± 9.31
F11	−1	−1	−1	−1	736.2 ± 1.62	0.479 ± 0.02	−11.8 ± 3.50	62.23 ± 5.98
F12	1	−1	1	−1	245.0 ± 1.16	0.237 ± 0.04	−10.3 ± 2.05	46.44 ± 2.74
F13	1	1	−1	−1	280.8 ± 3.07	0.262 ± 0.05	−12.3 ± 2.08	93.33 ± 4.87
F14	−1	1	1	1	237.0 ± 3.60	0.445 ± 0.07	−11.1 ± 1.95	72.60 ± 2.09
F15	−1	1	−1	−1	478.5 ± 2.76	0.815 ± 0.04	−11.0 ± 1.08	64.29 ± 5.87
F16	−1	−1	1	1	262.1 ± 2.84	0.348 ± 0.04	−10.9 ± 1.10	75.91 ± 4.21

Data optimization and model validation: A design space was established based on the product desirability. An optimized formula (O_1) was prepared as suggested by the program and was evaluated, and compared with the expected results.

2.2.4. *In-vitro* Drug Release

In-vitro drug release study was tested using dialysis membrane method in PBS at pH 7.4. The optimized formula was compared to the standard Rsv in distilled water (both containing 10 mg Rsv), where the samples were withdrawn over a period of 72 h [10].

2.2.5. *In-vivo* Pharmacodynamics Study

The *in-vivo* study was conducted on 24 male Wistar rats (170–200 g), which were allowed free access to water and food [11]. The rats were divided into 2 dietary groups. NFD group consisted of 6 rats, which were fed on normal fat diet (NFD), and HFD group consisted of 18 rats, and were fed on high fat diet (HFD). This diet regimen was continued for 6 weeks, and at week 7, the rats were fasted, anesthetized and blood samples were withdrawn to measure triglycerides (TGs) and total cholesterol (TC) [12].

Animals were then grouped into 4 groups, the first group; negative control rats, which were NFD group and received plain sodium carboxymethyl cellulose aqueous solution. The second group was the hyperlipidemic positive control group, which were HFD groupin which the rats recieved plain sodium carboxymethyl cellulose aqueous solution, while the third group were HFD group receiving Rsv in sodium carboxymethyl cellulose aqueous solution, and the last group was HFD group receiving the optimized formula (O_1). After 2 weeks, animals were then anesthetized and fasting blood samples were taken to measure TGs, TC, high-density lipoprotein (HDL) and low-density lipoprotein (LDL) [12].

3. Results and Discussion

3.1. Quality Target Product Profile and Risk Analysis

Potential causes of each of the CQA were outlined using Ishikawa diagrams, as represented in Figure 1.

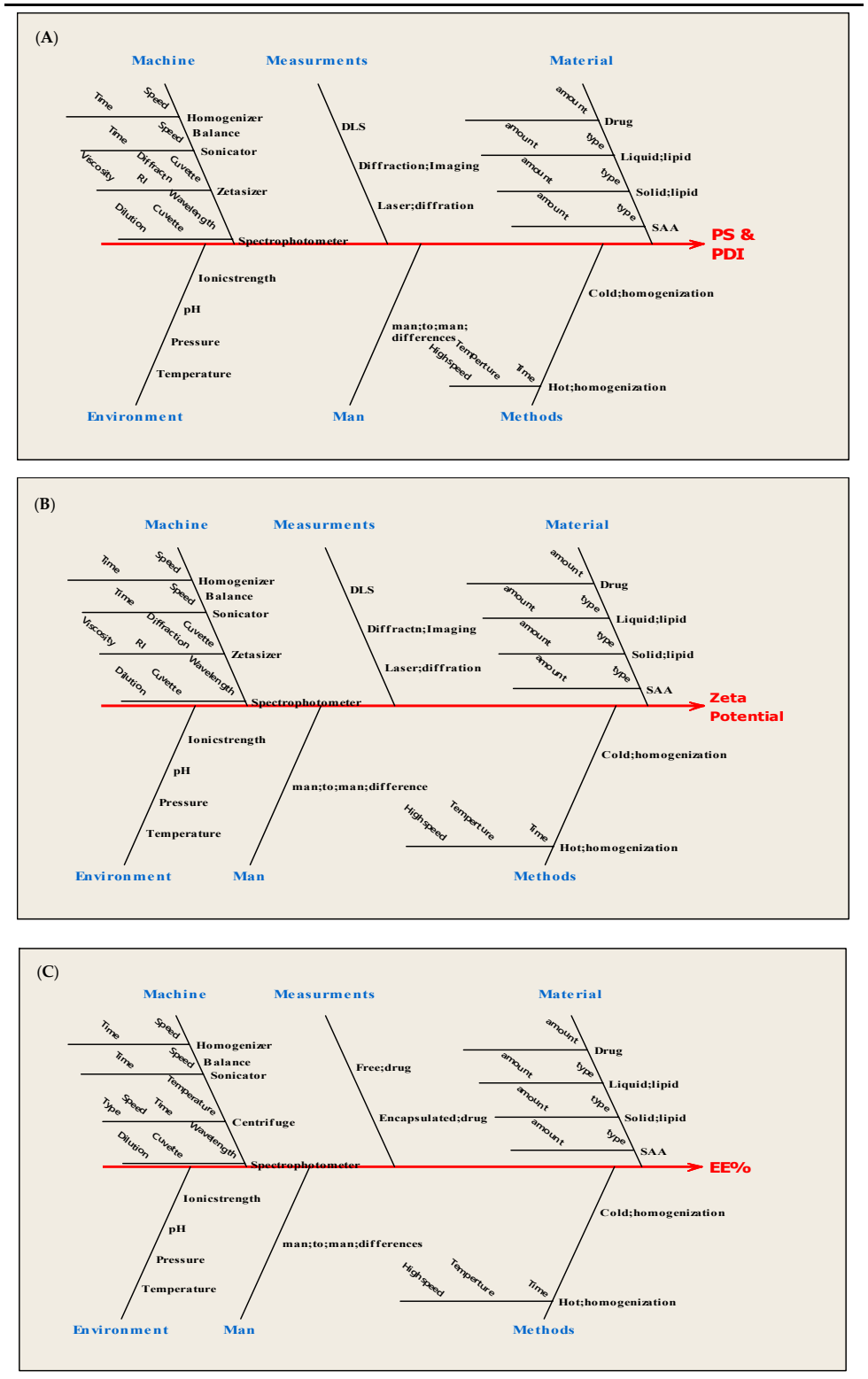

Figure 1. Ishikawa diagrams for different CQA; (**A**) PS and PDI, (**B**) ζ- pot and (**C**) EE%.

3.2. Screening of Different Solid Lipids, Liquid Lipids and Surfactants (SAA) for Nanovesicular Formulation

Rsv was found to be most soluble in Precirol® ATO 5 among the solid lipids, in oleic acid among the liquid lipids and in Tween® 20 among the SAA. Accordingly, these were the ingredients of choice for the nanovesicular preparations. The high solubility of Rsv in Precirol® ATO 5 may be due to the highly porous structure ofPrecirol® ATO 5 which allows more drug accommodation and solubility. The presence of methane sulfonamide hydrophilic moiety in Rsv resulted in imparting a slight

hydrophilic nature, which in turn lead to its better solubility in Tween® 20, than Tween® 80 [13]. However, the hydrophobic nature of the drug will allow the Tween® 20 to solubilize it more than Poloxamer® 188 [10].

3.3. Response Surface Design Analysis

Further analysis using ANOVA, indicated that all models were significant with significant effect of CPP/MA on the measured CQA at ($p < 0.05$).

3.3.1. Particle Size Analysis

The effect of the CPP/MA on the PS can be described as

$$PS = 361.1 - 67.6*X_1 - 44.7*X_2 - 57.6*X_3 - 6.2*X_4 + 20.6*X_{12} + 82.7*X_{13} + 31.3*X_{14} + 55.9*X_{23} - 43.1*X_{24} + 7.6*X_{34} \quad (1)$$

An increase in X1 or X2 resulted in a significant reduction in the particle size. Probably, this may be due to the reduction in the surface tension, when SAA% increases, making the oil droplets smaller. In addition, SAA would be able to coat the oil droplets, which would stabilize the dispaersion [4]. A larger particle size was observed when X3 was decreased, i.e., NLC was prepared, which may be attributed to the presence of the liquid lipid which might increase the hydrodynamic diameter [14]. The effect of the drug amount was insignificant.

3.3.2. PDI Analysis

The effect of the CPP/MA on the PDI can be described as

$$PDI = +0.41 - 0.11X_1 + 0.051 * X_2 + 9.344E - 003 * X_3 - 0.048 * X_4 + 0.053 * X_{13} - 0.067 * X_{24} \quad (2)$$

A lower lipid content (X_1) resulted in a higher PDI value, which may be due to the insufficient amount of lipid to enclose Rsv, leading to the heterogencity of the system [15]. The PDI was bigger at high SAA%, which could be attributed to the excees amount of SAA that may accumulate on the surface of the vesicles, rsulting in increasing the system's heterogencity [8]. Moreover, the excess SAA might lower the surface tension to an extreme extent, which might rupture the vesicles and increase the system's heterogenicity [4]. A higher drug amount had a statistical effect on the PDI, while the ratio between soild lipid and liquid lipid was insignificant.

3.3.3. Zeta Potential Analysis

The effect of the CPP/MA on the ζ-pot can be described as

$$\zeta\text{-pot} = +14 - 1.10 * X_1 + 0.80 * X_2 + 1.51 * X_3 - 0.98 * X_4 - 0.56 * X_{13} + 0.92 * X_{23} - 1.18 * X_{34} \quad (3)$$

The increase in the ζ-pot by decreasing X_1, could be due to the reduction in the particle size by the increase in the total lipid content, which in turn reduces the surface area of the vesicles with less charge accommodation [16]. Moreover, the ζ-pot was increased by the increase in the SAA%, which could be due to molecular polarization and the adsorption of the surface acting agent in water. The adsorbed SAA in water could be absorbed to the emulsifier layer of particle/water interface and form an electric double layer that is similar to ionic state [17].

3.3.4. EE% Analysis

The effect of the CPP/MA on the EE% can be described as

$$EE\% = 76.46 + 4.33 * X_1 - 6.25 * X_2 - 5.02 * X_3 + 5.38 * X_4 - 4.49 * X_{13} + 4.61 * X_{23} - 5.63 * X_{24} \quad (4)$$

A significant increase in the EE% was observed with the increase in the lipid content, which might be due to sufficient amounts of lipids that can be used to encapsulate the drug [15]. Moreover, the higher SAA% resuted in a lower EE%, which may be due to the disruption of the vesicules at high SAA concentration [4]. When NLC was prepared the EE% increased, which could be due to the

incorporation of a liquid lipid into a solid lipid, leading to a number of crystalline sequence disturbances and defects in the crystal lattice, which could in turn create a space for the encapsulation of Rsv molecules [10]. Finally an increase in the EE% was observed by the increase in the drug amount, which may be due to the more availability of the Rsv, which consequently improves its retention within the vesicles [17].

3.4. Model Validation, Data Optimization and Control Strategy Establishment

An optimized formula (O_1), with desirability 0.893 was prepared (Table 2). The validity of the design was established by comaring the observed results with the expected ones, which were found to statistically insignificant. A successful design space was established with the control space that ensures the product reproducibility.

Table 2. Composition of the optimized formula with the expected and the observed results.

CPP/MA	Level in Coded Value	
Total lipid content (X_1)	+1	
SAA% (X_2)	−0.623	
SL: LL ratio (X_3)	−1	
Drug amount (X_4)	+0.992	
CQA	**Results**	
	Expected	**Observed**
PS (nm)	**352.345**	**310.5**
PDI	**0.259**	**0.243**
ζ-pot (mv)	**−20.803**	**−24.7**
EE (%)	**94.663**	**93.87**

3.5. In-vitro Drug Release Analysis

NLC optimized formula was able to release the drug in a sustained manner as compared to the standard Rsv (Figure 2). The optimized formula was able to sustain the release for up to 72 h. The drug on the surface of the vesicles resulted in an initial burst release which was followed by a sustained release pattern [18].

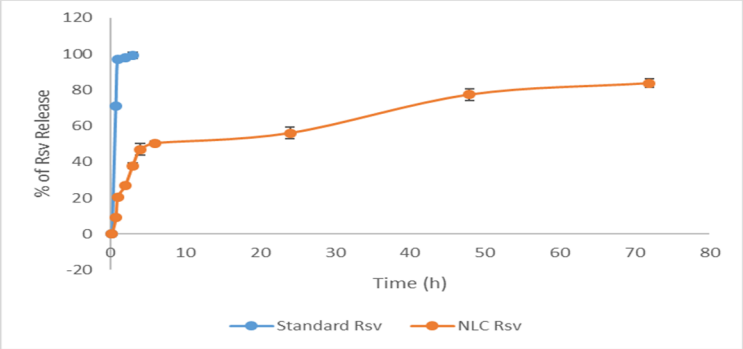

Figure 2. In-vitro release of Rsv from the optimized Rsv formula and standard Rsv.

3.6. In-vivo Pharmacodynamics Study

In agreement with previous reports [19] the hyperlipidemic positive control group exhibited a significant increase in TGs, TC and LDL when compared to the negative control. Compared to standard Rsv, the optimized formula O_1 significantly reduced serum TC level by 26.6% and LDL by 46%. The elevation in HDL (13.95%) and reduction in TGs (39%) were not significant.

Statins were reported to have side effects among a quite significant number of patients (5–20%) with more side effects appearing at higher doses [20]. The use of nanovesicular formulation of Rsv

may increase its solubility, and hence its bioavailability. Moreover, the lipid formulation could induce bile secretion in the small intestine where the NLC would be associated with the bile salts, forming mixed micelles, and thus the NLC would go to the lymphatic circulation directly, bypassing the first pass effect, and thus promoting its better absorption [21]. All this resulted in improving and sustaining the antihyperlipdemic activity of Rsv NLC when compared to the standard Rsv.

4. Conclusions

QbD approach was found to be a very useful approach the in formulation of ananovesicular carrier loaded with Rsv. Several tools have been used as the risk assessment and design of experiments in the screening and the optimization of the nanovesicular carriers. A design space was established which defines the control strategy for the formulation of Rsv-nanovesicular carrier. This control strategy gives the permitted ranges of the total lipid content, SAA%, type of the nanovesicle and the drug amount, that produced the nano-vesicle with optimal PS, PDI, ζ-potential and EE% for any further studies. The prepared optimized formula managed to significantly lower each of the TC, TGs and LDL and to elevate the HDL as compared to the positive control, thus proving the potential use of Rsv-NLC as a successful anti-hyperlipidimc agent. A full successful practical use of QbD approach in pharmaceutical development was applied by the use of several advanced techniques, which could be considered a reliable reference for further nanovesicular carriers' formulations.

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