RESEARCH ARTICLE

Chemometric approach for developing nanostructured self-emulsifying drug delivery systems of rosuvastatin calcium containing a dietary lipid with improved biopharmaceutical performance

Jagdish Kumar Arun[1], Rajeshwar Vodeti[1, 2*], Birendra Shrivastava[1], Vasudha Bakshi[2]

[1] School of Pharmaceutical Sciences, Jaipur National University, Jagatpura, Jaipur, Rajasthan-302 017, India

[2] School of Pharmacy, ANURAG Group of Institutions, Venkatapur (V), Ghatkesar (M) Medchal (Dist.), Hyderabad, Telangana-500 038, India

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ABSTRACT

Objective(s): The present work deals with the formulation of a nanostructured self-emulsifying delivery system of rosuvastatin calcium-containing a dietary lipid by chemometric principles to improve the biopharmaceutical performance of the drug. Excipient screening was performed based on the solubility of drug and phase behavior study using an aqueous titration method. A blend of liquid lipids, emulgents, and co-emulgents used to prepare the self-emulsifying delivery systems of the drug. Methods: Systematic optimization of the composition of the different formulation of nanostructured self-emulsifying drug delivery system (NSEDDS) performed using optimal experimental study design and characterized for emulsification time, zeta potential, globule size, polydispersity index, and drug release. In vitro drug release in simulated fluids and in vivo pharmacokinetics in rats were performed to compare the biopharmaceutical performance. Results: The optimized NSEDDS contained 15% of lipid (Isopropyl myristate), 75% of emulgent (Tween 20) and 10% of co-solvent (Ethanol), which exhibited fast emulsification in 150 seconds, globule size of 68 nm, zeta potential of 27 mV and more than > 85% drug release within 30 minutes. Conclusions: In vivo pharmacokinetic study in the rat model suggested that improvement of AUC and Cmax values of 4.89 to 4.45-folds, whereas reduction of Tmax value of 0.95-folds in case of NSEDDS than pure drug. Finally, the optimized nanostructured self-emulsifying formulation of rosuvastatin calcium showed greater effectiveness in enhancing the dissolution and absorption performance of the selected model drug.

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INTRODUCTION

Rosuvastatin calcium (RVC), a potent antihyperlipidemic agent, inhibits the synthesis of hexamethyl glutaryl reductase coenzyme-A required for cholesterol biosynthesis in the body. It has been reported to be very effective over atorvastatin and other statins with respect to their therapeutic performance. Despite its excellent efficacy, RVC encounters multiple challenges concerning its biopharmaceutical characteristics and the rate-limiting reasons such as poor aqueous solubility, poor oral bioavailability, limited intestinal permeability, and moderate to high hepatic first-pass metabolism, etc. [1, 2]. Although numerous novel drug delivery systems have been employed for addressing the above challenges, yet the quest for developing the novel, effective and cost-effective delivery systems remains open.

SEDDS (Self-emulsifying drug delivery

* Corresponding Author Email: *Email*

systems) defined as an isotropic mixture of the selected drugs dissolved in the blends of lipid, emulgent, and cosolvents, which upon emulsification in the presence of aqueous phase produces nanoemulsion with particle size less than 250 nm [3]. Such formulations are easy to prepare, highly stable, involves low manufacturing cost, and provides a distinct advantage for improvement of biopharmaceutical performance of drugs belongs to BCS class II and IV. Most of the drugs formulated in the form of SNEDDS for augmenting their biopharmaceutical performance. For RVC, especially some literature reports are available on SNEDDS, but still, a moderate improvement in oral bioavailability was observed [4].

The application of principles of Quality by Design (QbD) for systematic optimization of pharmaceutical drug products has become a trend and regulatory requirement. In this regard, the use of chemometric multivariate tools like experimental designs is highly beneficial in establishing cause and effect relationship among the CPP (critical process parameters), and dependent variables. Apart from conventional formulations, experimental models have been highly useful in producing optimized product performance for the nano pharmaceutical formulations [5].

In the current research, the effort implemented for the development of RVC-NSEDDS of using the natural dietary lipids. The prepared formulations were systematically optimized using a chemometric approach. Using mixture design, SNEDDS containing an isotropic mixture of excipients optimized for a complete understanding of the product and process parameters for attaining consistency in the product quality attributes. NSEDDS was further evaluated through a series of in vitro and in vivo studies to ratify the improvement of the optimized batch's biopharmaceutical performance compared with pure drug.

MATERIALS AND METHODS

RVC was supplied from Mylan Ltd. (Vishakhapatnam, India). Various dietary oils, emulgents and cosolvents used for the formulation development were purchased from Fischer Scientific and S.D. Fine Chemicals (Mumbai, India).

Defining the formulation objectives

The formulation objectives were defined for preparing the NSEDDS formulation of RVC, which include the key characteristics of the formulation as high drug solubility, faster dissolution rate, improved drug absorption rate into the systemic circulation, and enhanced activity for reducing the elevated levels of biomarkers of hyperlipidemia.

Identification of the formulation quality attributes

To improve the biopharmaceutical performance of the selected drug, critical quality attributes of the developed formulations were identified based on framed objectives. The emulsification time, zeta potential, globule size, and in vitro drug release of the formulation selected as quality attributes parameters based on their direct impact on the end-product quality and performance.

Formulation risk assessment and management

In order to identify the critical formulation and process parameters, the impact assessment was carried out with the help of Ishikawa fish-bone diagram. All the parameters belonging to the categories such as men, material, machine, measurement and milieu influencing the critical quality attributes of the RCV-SNEDDS. Further, a quality risk matrix was generated by listing all the key formulation attributes and assigning color coding (Green, yellow and red) to them based on the criticality of their impact on the critical quality attributes [6-8]. Only the formulation attributes with high and medium risk were taken into analysis further through factor screening study.

Screening of the excipients

The solubility studies of the drug-using palm oil, corn oil, jojoba oil, flax-seed oil, and olive oil (as dietary lipids), tween 20, 40, and 80 (as emulgents), propylene glycol and polyethylene glycol 400 (as co-solvents) is the primary identification method to evaluate the screening of the excipients using electrical shaker containing a water bath maintained at 37 ± 0.5°C for 24 h with the addition of an excess quantity of the selected drug. Later with a specific time point, visually examined the solubility of drug in vials and, if required, excess adds drug. After that, vials were placed in the centrifuged tubes, and supernatant fractions were collected, and the drug was extracted in methanol. In each of the excipients, drug content was analyzed by UV-Visible spectrophotometer at 238 nm, and solubility was reported in mg/mL.

Construction of ternary phase diagram

Both water and oil titration methods were used for identifying the phase compatibility of

the excipients with maximal solubility of the drug. Various ratios of emulgents and cosolvents (1:1, 2:1 and 3:1) were prepared, and mixed with lipid in ratio 1:9 to 9:1. Titrations were performed by considering the nanoemulsion formation as the end-point to delineate the boundaries of nanoemulsion region [9].

Formulation and optimization of the RVC-SNEDDS

RVC-SNEDDS formulation was prepared by solubilization of the drug with selected oil and then step-wise blending with emulgent and co-solvent at 37°C on a hot plate and magnetic stirring for 30 min to obtain a homogenous isotropic mixture and it stored in a cool and dry place [10]. The obtained SNEDDS formulation was optimized using a chemometric-based experimental design that is D-optimal mixture design (User-defined; Design Expert® 9.0.1 software) with 3 factors and 3 levels and obtained 16 experimental trial formulations. The independent factors, such as the amount of lipid, emulgent, and co-solvent, were selected at low (-1), medium (0), and high (+1) levels, as CQAs for SNEDDS formulations [11, 12].

Characterization of the RVC-SNEDDS

Emulsification time

The prepared formulations were poured in distilled water, and the total time taken by the formulation to get dispersed entirely in water was noted as emulsification time.

Zeta potential and globule size

The obtained formulations were diluted using distilled water in 100-folds and determined the globule size (in nm) and zeta potential distribution (mv) using zeta-sizer ZS-90 (Malvern Instruments, UK).

In vitro drug release

The dialysis bag technique was used for the determination of in vitro drug release as reported literature. The prepared SNEDDS formulation (1 gm) was filled in a dialysis bag and subjected to release study using 0.1 N HCl containing 0.5% sodium lauryl sulfate as the release medium. The study duration was for the period of 2 h, and aliquot samples were collected at an interval of 15, 30, 45, 60, and 120 min, respectively [10, 13, 14]. A new SNEDDS formulation without drug and the pure drug was also subjected to release study, and samples were collected on the time as mentioned above points to nullify the effect of formulation excipients [15]. The drug content in the samples was determined as per validated reported method for the pure drug, and cumulative percent drug release versus time was calculated [16-18].

Chemometric data analysis and selection of optimum formulation

Using the chemometric principles, mathematical modelization of the experimental data obtained from the sixteen trial formulations of RVC-SNEDDS was conducted with the help of multivariate regression analysis. A quadratic model fitting was attempted for each of the critical quality attributes. Model fitness was evaluated from the parameters such as ANOVA model significance, correlation coefficient, lack of fit, and predicted residual error sum of squares [19, 20]. 3D maps obtained for each of the critical quality attributes of the developed formulation. The 2D and 3D graphs analyzed as per the selected independent and dependent variables and their relationship and its impact on the end-product quality. The optimum formulation was chosen by mathematical and graphical search methods, based on faster emulsification, smaller in globule size, higher in zeta potential, and faster in vitro drug release characteristics [21].

Evaluation of the optimized RVC-SNEDDS

The following characteristics for the optimized formulation, such as final globule size, zeta potential, in vitro drug release, and transmission electron microscopy (TEM), were determined.

Pharmacokinetic study

The pharmacokinetic study was performed and carried out as per the approved Institutional Animal Ethical Committee (Protocol number: I/IAEC/AGI/025/2018), Anurag Group of Institutions, Hyderabad, India and CPCSEA, Government of India. The study's objective is to evaluate the enhancement of oral bioavailability of the administered optimized batch of RVC-SNEDDS and pure drug suspension. The two groups (I and II) of male Sprague-Dawley rats, each of six animals were randomly distributed, and the optimized liquid formulation of SNEEDS was administered orally (40 mg/kg body weight of rat) to the Group I, and pure drug suspension (40 mg/kg body weight of rat) to the group II animals. Soon after dosing, blood samples (~0.2 mL) were withdrawn from the

Table 1: List of formulation objectives framed for RVC-SNEDDS

Objectives type	Target objective	Justification for objective selection
Dosage form	SNEDDS	Selection of lipid-based self-nanoemulsifying system helps in the oral bioavailability enhancement of poorly bioavailable drug, RVC
Dosage type	Immediate release	Faster onset of action leads to enhanced therapeutic benefits
Dosage strength	40 mg	Unit dose of RVC incorporated in a single formulation of SNEDDS
Route of administration	Oral	Recommended route for delivery of RVC for the management of hypertension
Drug content	100%	Ideal value for the RVC-SNEDDS containing
Pharmacokinetics	Higher C_{max} and AUC	Required for achieving higher drug levels into the systemic circulation for enhanced therapeutic action

Table 2: List of critical quality attributes of RVC-SNEDDS and justifications for them

Quality attributes	Target	Is this critical?	Justification for selection of quality attributes
Emulsification time	<5 min	Yes	Lower values of emulsification time facilitate the formation of nanoemulsion; hence was taken up as highly critical.
Globule size	<100 nm	Yes	Smaller globule size allows easier penetration through gastrointestinal epithelial lining and paracellular pathways; hence was regarded as highly critical.
Zeta potential	- 40 to 40 mV	Yes	Negative and positive values of the zeta potential are required for stabilizing the nanoemulsion globules; hence was regarded as highly critical.
%Drug release	100%	Yes	It indicates performance of the dosage form during early dissolution phase; therefore, was considered as critical.

tail vein by puncturing, and plasma separated by centrifugation method plasma and drug analysis by HPLC as per reported literature [16]. The plasma concentration of the drug was estimated at different time intervals and non-compartmental analysis used for the determination of pharmacokinetic parameters using the software of Kinetica 5.0 (Thermo Fisher Scientific, USA).

Statistical data analysis

The Prism 6.0 software (GraphPad Inc., USA). was used for the analysis of variance (ANOVA) statistical obtained data was interpreted using post-hoc analysis and Student's t-test at the level of significance of 5%.

RESULTS AND DISCUSSION

Defining the formulation objectives

Table 1 gives an account of the list of formulation objectives for the RVC-SNEDDS, including formulation design, dose requirement, route of administration, drug content, drug release, pharmacokinetics, stability, and packaging parameters.

Identification of the CQAs

Table 2 gives an account of the formulation of critical quality attributes (CQAs), directly linked with the critical formulation attributes of the RVC-SNEDDS. Emulsification time was considered to be highly important for faster emulsification of the prepared formulation. Globule size and Zeta potential were selected due to its direct impact on dissolution rate and drug absorption and its effect on the prepared formulation's stability. Drug release is pivotal for faster drug absorption into the systemic circulation.

Excipients Screening

The solubility data of RVC in different lipids in the order as follows: corn oil < olive oil < jojoba oil < flaxseed oil < palm oil>. Corn oil was chosen as the lipid with maximum solubility for the drug, as shown in **Supplementary Data Fig. S1**. Similarly, **Supplementary data Fig. S2** indicates the bar chart depicting the solubility of the drug in emulgents and cosolvents. The order is as follows: Tween 20 < Tween 40 < Tween 80, while solubility profile in cosolvents was in the order: PEG 200 < propylene glycol.

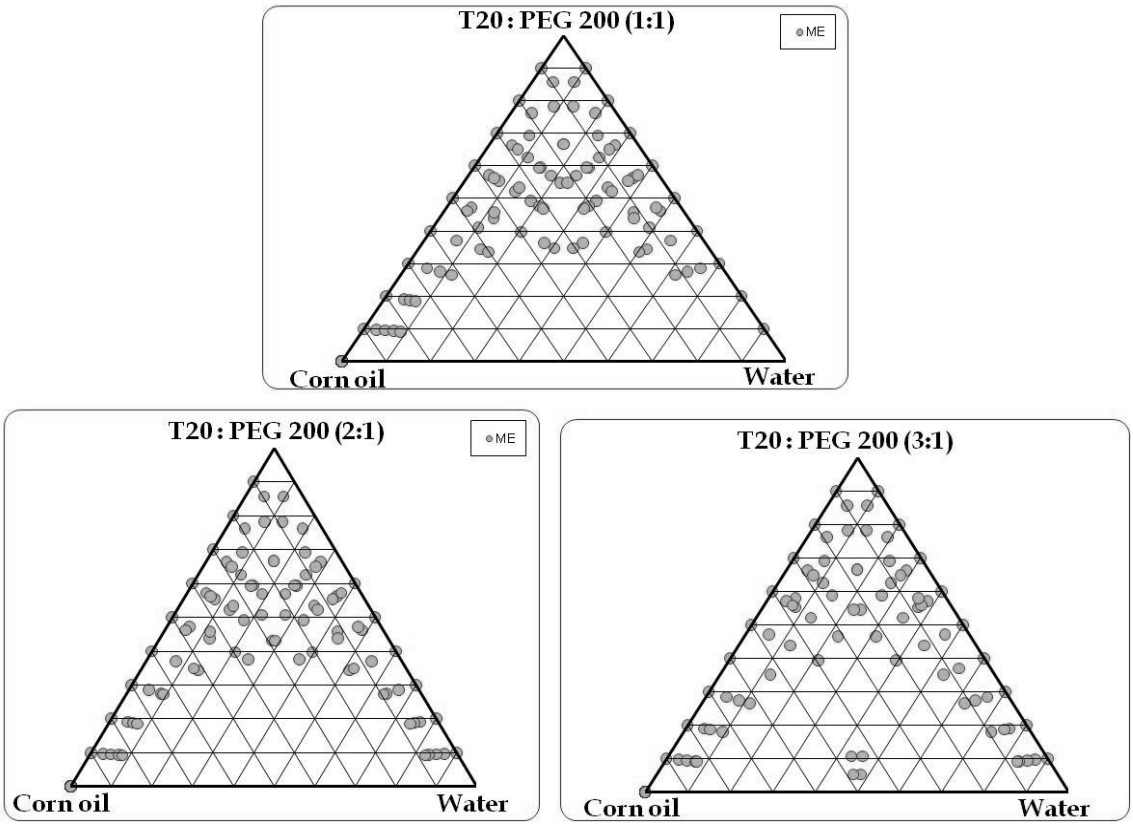

Fig. 1: Pseudoternary phase diagrams depicting nanoemulsion region with S_{mix} ratios 1:1, 2:1 and 3:1

Construction of ternary diagrams

The pseudo-ternary phase diagrams for corn oil along with Tween 20 and PEG 200 (S_{mix}) at 1:1, 2:1, and 3:1 ratio by titration method, as shown in **Fig. 1**. A right nanoemulsion region was observed due to the appropriate emulsification of the lipidic phase in the selected surfactant and cosolvent as per the prepared S_{mix}. Moreover, increasing the amount of emulgent in the S_{mix} ratio showed no substantial change in the nanoemulsion area in the ternary diagrams. Thus, the S_{mix} ratio 1:1 was selected for preparing the RVC-SNEDDS.

Characterization of liquid SNEDDS

The obtained 16 experimental trials of RVC-SNEDDS formulations were prepared as per the applied experimental design and selected the critical quality attributes values as shown in **Table 3.**

Emulsification time

The emulsification time of the RVC-SNEDDS found to be ranging from 109 and 194 seconds, which shows faster emulsification of the prepared formulations [22].

Globule size and zeta potential

The globule size data of the prepared RVC-SNEDDS formulations are shown in between 48 to105 nm, which indicated that the globule size of all the formulations was less than 250 nm to become declared as the nanostructured nature of the system. The zeta potential of the prepared RVC-SNEDDS formulations is shown in between - 23 to - 45 nm, which confirms that all the prepared formulations are stable [23].

In-vitro drug release study

The in vitro drug release profile of RVC SNEDDS formulations is shown in **Fig. 2**. The obtained in vitro data and plotted graphs showed slower drug release as compared to RVC-SNEDDS formulations. In-vitro drug release study performed for 2h, RVC-SNEDDS showed nearly 98% drug release, while only 28% drug was released from the pure drug suspension in the studied period. This

Table 3: Design matrix indicating composition of RVC-SNEDDS as per the D-optimal mixture design

*Formulation code	Critical formulation parameters		
	Corn oil (mg)	Tween 20 (mg)	PEG 200 (mg)
F1	250	500	250
F2	276	446	278
F3	250	450	299
F4	281	491	228
F5	300	400	300
F6	300	424	276
F7	300	500	200
F8	300	450	250
F9	233	485	282
F10	250	450	299
F11	200	500	300
F12	300	450	250
F13	250	450	299
F14	250	500	250
F15	200	500	300
F16	271	473	256

Total weight of all formulations are constant (1 gm)

indicated nearly 3.5-folds enhancement of drug release from the SNEDDS formulation compared to the pure drug suspension [24, 25].

Chemometric analysis of data and response surface mapping

Using chemometric principles, obtained experimental values obtained were subjected to mathematical modelization and statistical evaluation of the model fitness. Quadratic and cubic model fitting was observed, which also showed the presence of interaction between the studied factors. The obtained polynomial equation coefficients as per the model shown in Equation (1) for the critical quality attributes.

$$\mathbf{Y = \beta 1X1 + \beta 2X2 + \beta 3X3 + \beta 4X1X2 + \beta 5X2X3 + \beta 8X1X2(X1\text{-}X2) + \beta nXnXm(Xn\text{-}Xm)} \quad (1)$$

Where, Y specifies the CQA; β1 to β5 are the model terms coefficient values; X1, X2, and X3 designates the optimization CMAs.

Model suitability was confirmed from high values of correlation coefficients for the critical quality attributes ranging between 0.948 to 0.998. The insignificant values of lack of fit indicated goodness in the model fitting [26]. **Fig. 3** illustrates the 2D and 3D-response surface plots for various critical quality attributes, where intricate patterns and highly curved nature of the plots indicate a high degree of interaction among the studied factors. On emulsification efficiency, both emulgent and cosolvent concentration showed maximal influence, while the level of lipid and emulgent maximally influenced globule size and zeta potential. Drug % release showed maximal impact by lipid and emulgent, with mild influence by cosolvent too.

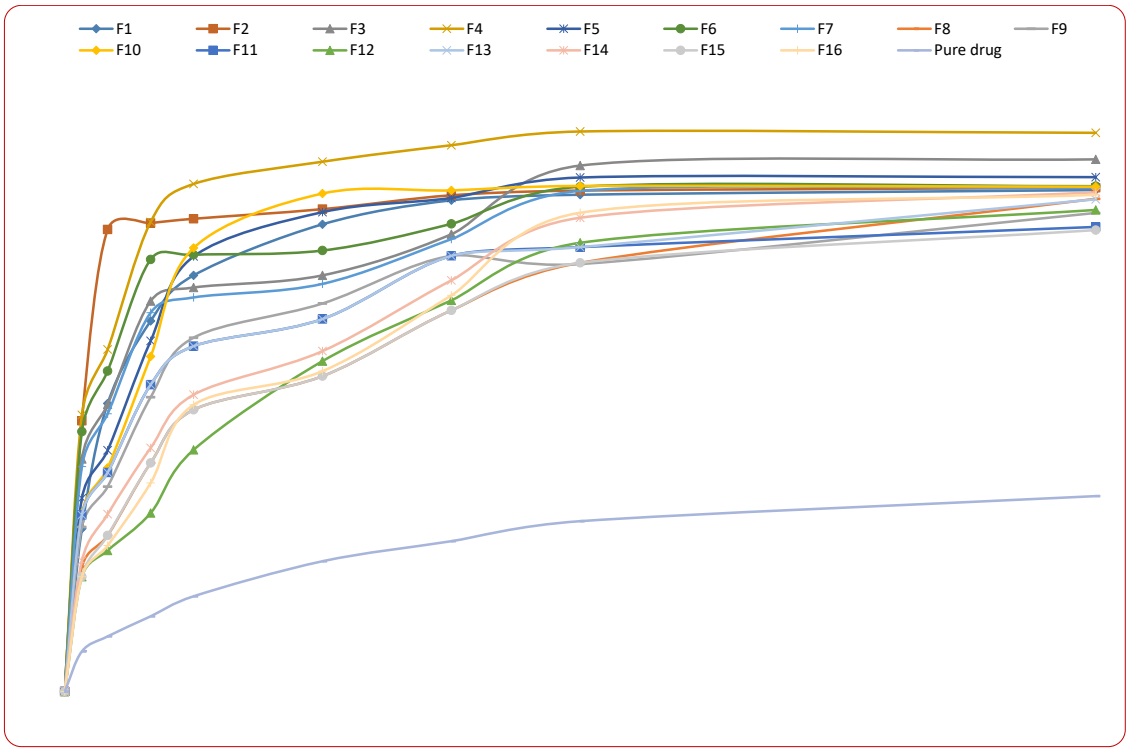

Fig. 2: Cumulative percent drug release from different RVC-SNEDDS; Data expressed as Mean ± SD (n=3); [RVC: Rosuvastatin calcium]

Selection of the optimum RVC-SNEDDS formulation

The optimum formulation was identified based on various goals, i.e., faster emulsification time, small globule size, high zeta potential, more rapid and maximal drug release for the prepared RVC-SNEDDS formulation. The desirable region was selected based on overlay plots (Fig. 4.) The best formulation composition includes corn oil (271 mg), Tween 20 (473 mg): Transcutol HP (256 mg), as specified by the selected point in Fig. 4, along with the standards of all the critical quality attributes of the RVC-SNEDDS.

Evaluation of RVC-SNEDDS optimized batch

Globule size and zeta potential

The mean globule size and zeta potential data of RVC-SNEDDS optimized formulation were found to be 69 nm and 27 mV. Besides, the positive magnitude of the zeta potential confirmed thermodynamically stable nature developed formulation with obtained desired nanosize formulation (Fig. 5).

Transmission electron microscopy (TEM)

The TEM image of RVC-SNEDDS optimized formulations diluted in distilled water, where nanoemulsion globules are clearly visible under bright field imaging as shown in **Fig. 5**.

Pharmacokinetic study

Fig. 6 depicts the plasma concentration versus time profile of the drug from SNEDDS formulation and pure drug suspension after oral administration to the rats. The plasma concentration data from RVC-SNEDDS showed nearly 4.89 and 4.45-folds improvement in C_{max} and AUC0-24h ($p<0.0001$), and 0.98-fold reduction in the T_{max} as compared to pure drug suspension ($p<0.05$). This indicated distinct enhancement of drug absorption potential from the SNEDDS formulation owing to faster drug release rate and improvement in the intestinal drug absorption by avoiding hepatic first-pass metabolism as the plausible mechanisms reported in the literature [27-32].

CONCLUSION

The current effort construes active formulation development of the RVC-SNEDDS with improved biopharmaceutical performance. The use of chemometric principles helped in understanding

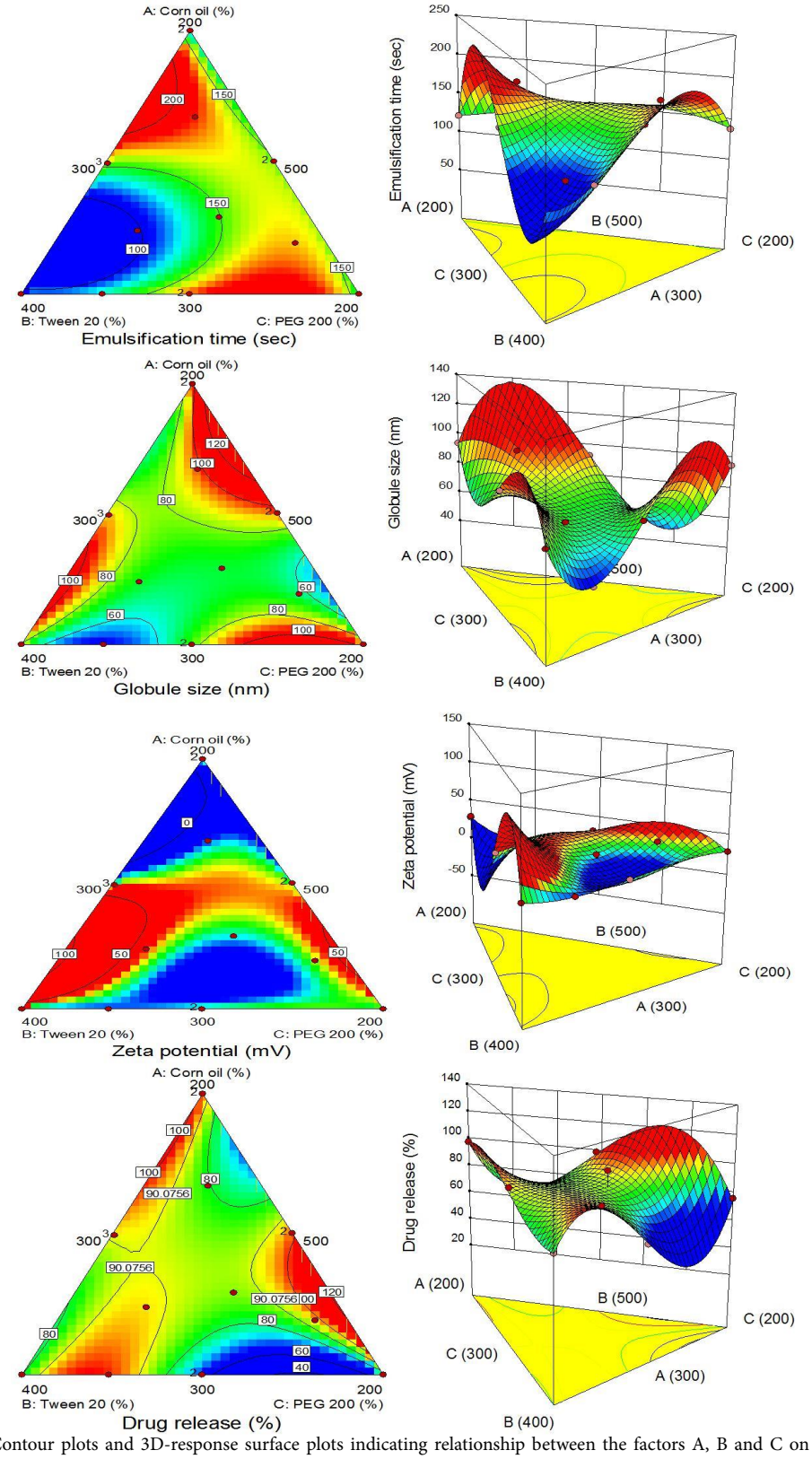

Fig. 3: 2D-Contour plots and 3D-response surface plots indicating relationship between the factors A, B and C on the response variables, emulsification time, globule size, zeta potential, %drug release of RVC-SNEDDS

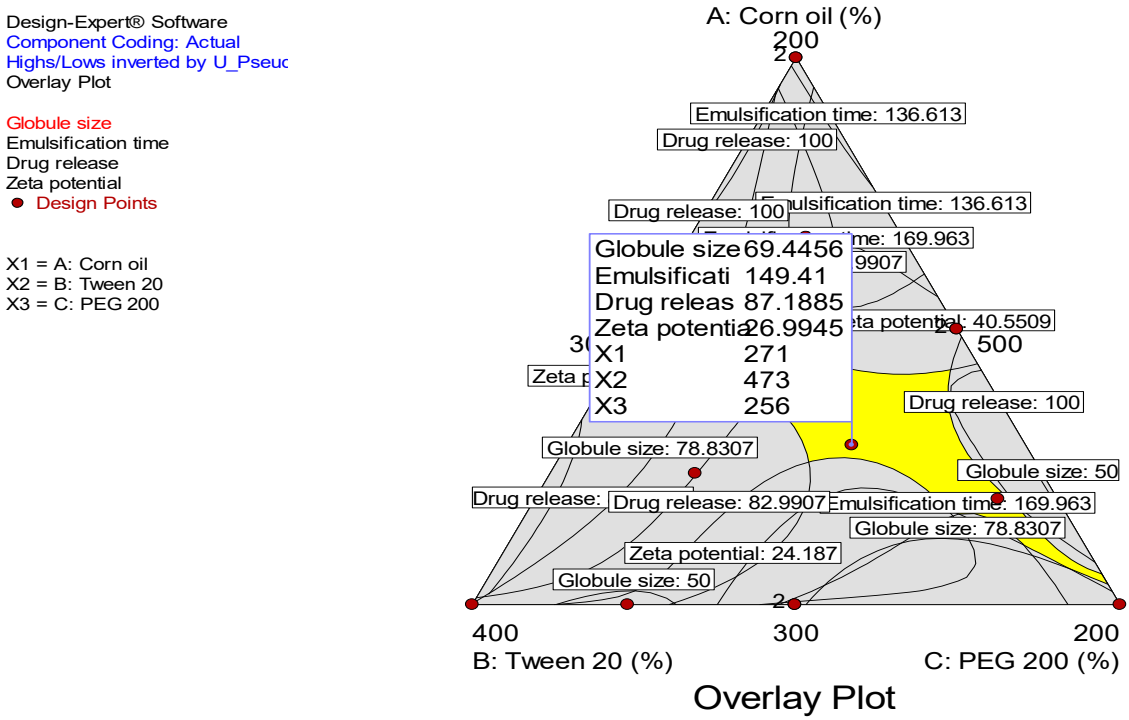

Fig. 4: Design space overlay plot for optimized RVC-SNEDDS

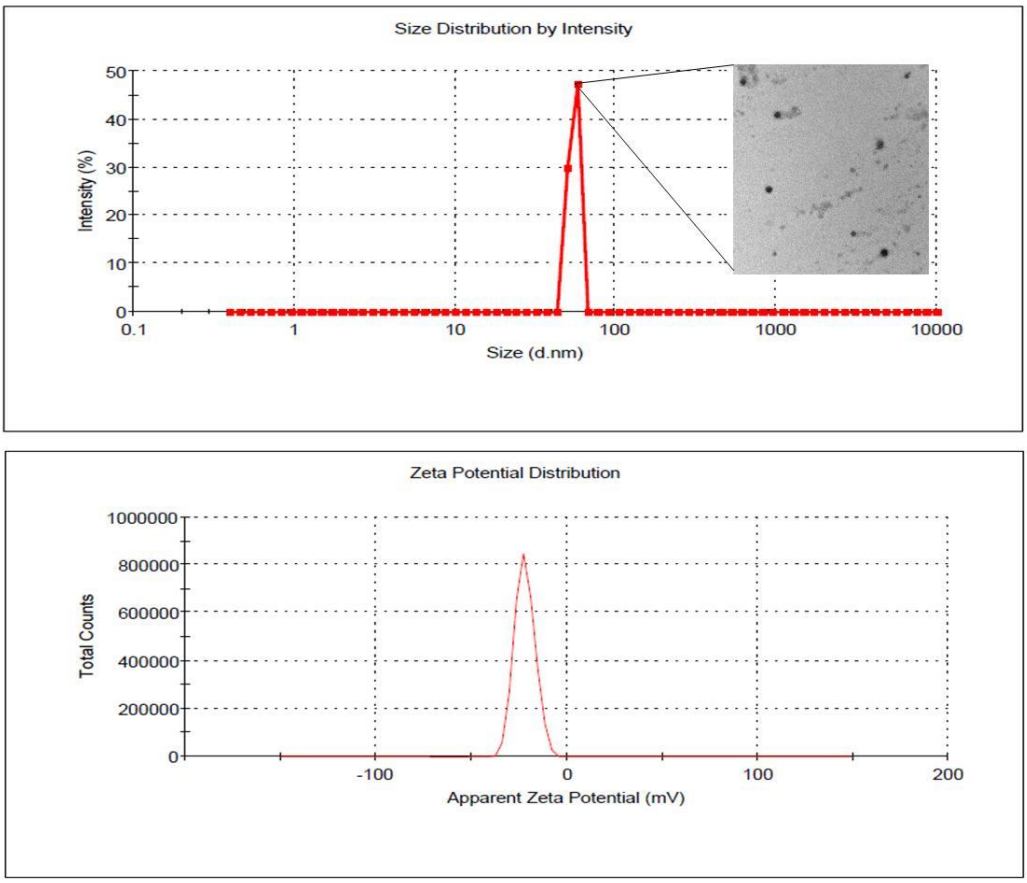

Fig. 5: Globule size distribution, zeta potential measurement and TEM image of the optimized RVC-SNEDDS

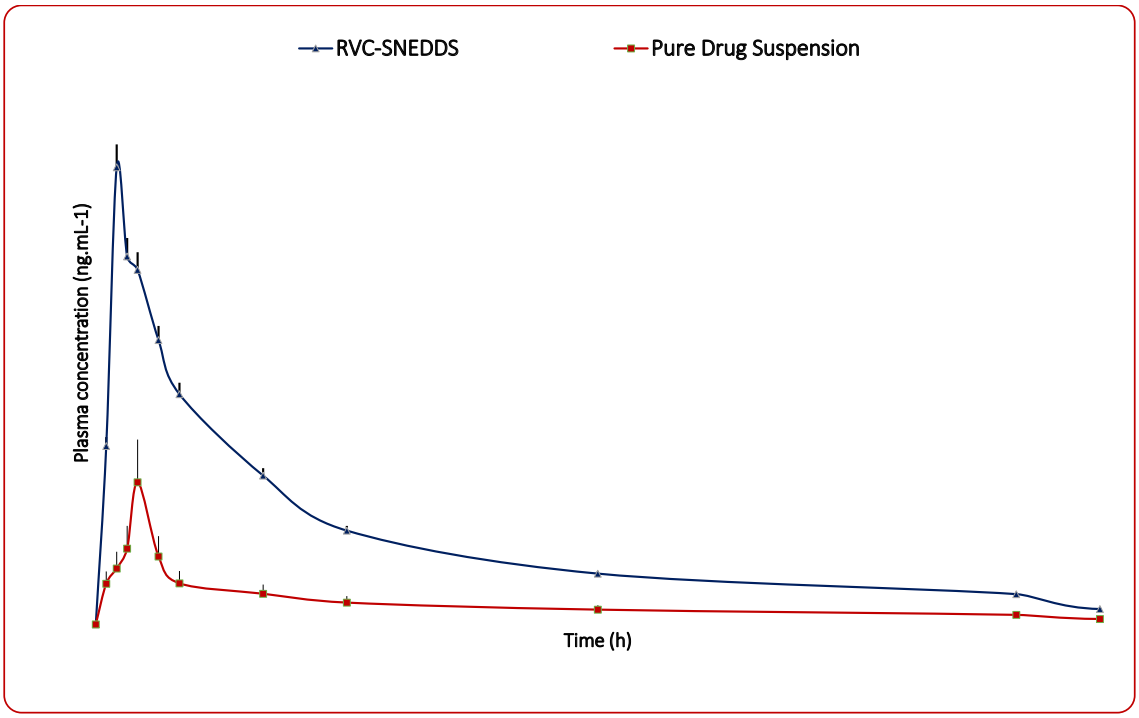

Fig. 6: Plasma concentration *versus* time profile of RVC from the optimized SNEDDS and pure drug suspension; Data represented as Mean SD (n=6)

the influential parameters and optimizing them with the multivariate experimental design approach. Thus, it can be clinched that RVC-SNEDDS established using selected polymers has industrial prominence concerning its applicability with oral bioavailability potential.

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COMPETING INTEREST

The authors have declared that there is no conflict of interest.

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