Nano-colloidal carrier *via* polymeric coating for oral delivery of isradipine

VIKASH KUMAR[1,*], HEMA CHAUDHARY[2], ANJOO KAMBOJ[3,*]

[1]Department of RIC, I.K. Gujral Punjab Technical University, Kapurthala, Punjab, India
[2]PDM College of Pharmacy, PDM University, Bahadurgarh, Haryana, India
[3]Chandigarh College of Pharmacy, Chandigarh, India
*Corresponding authors: Vikash Kumar; Department of RIC, I.K. Gujral Punjab Technical University, Kapurthala, Punjab 144603, India; Phone/Fax: +91 8950366343; E-mail: vikashruhilo1@gmail.com; Dr. Anjoo Kamboj; Chandigarh College of Pharmacy, Landran, Chandigarh, Punjab 140307, India; Phone/Fax: +91 9781925296; E-mail: anjookamboj@gmail.com

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Abstract: Our research objective was to develop, characterize, and optimize stable form of nano-colloidal carrier with Eudragit-coated solid lipid nanobioparticles (SLNbp) for oral delivery of isradipine (ISR). To achieve, a three factors, i.e., lipid-to-surfactant ratio (A, % w/w), Eudragit L100 (B, % w/w), and sonication time (C, minutes) at three levels (−1 and +1 levels of quality central level) was applied to develop SLNbp using response surface methodology at constant ratio of ISR and rutin. The second-order polynomial quadratic equations of responses [R1, R2, and R3; entrapment efficiency (EE), particle size, and drug release] were constructed and also plotted response surface (two- and three-dimensional) plots. The derived polynomial equation and 2D and 3D model were showed the relationship between the responses of the selected independent variables (A, B, and C). The model validation and optimization was performed by numerical checkpoint analysis to predict the optimized solid lipid nanobioparticle formulas (ONbp 1–10). The optimized formulations prepared and during evaluation ONbp 3 has better smaller particle size (106 nm), sustainable release (95.61% up to 40 h), higher EE (97.85%), and drug content (99.92% ± 0.08%) during 3-month storage showed good stability. Therefore, its performance can be considered for further development of stable oral drug delivery system of ISR.

Keywords: ISR, rutin, lipid, Eudragit, solid lipid nanobioparticles, oral delivery

Introduction

Even the most up-to-date oral drug delivery (ODD) or medication had one problem, i.e., it is difficult to control the time or release of the active pharmaceutical ingredient (API). After exceptional accessibility and patient compliance, ODD is a preferential conventional as well as accepted route as compared to other delivery systems. Besides this, ODD has some another significant limitations like bioavailability, stability, solubility, or poor permeability across gastrointestinal (GI) biological membrane or barrier. To resolve these problems, drug-loaded nanoparticles were developed [1–4], which have capability of easily absorbed and transit through GI environment and barrier. For this rationale, in the beginning of new century, an alternate colloidal carrier based on lipid [5–7] nanoparticulate system [8–11] was developed to minimize ODD limits. These particles are composed of physiologically tolerated lipids and surfactants plus co-surfactant where the drug is usually encapsulated in a core having diameter in nanometer. They are extensively being used to improve the drug solubility, absorption prolonged release, and maintain biological activity by minimize the degradation, metabolism, and least side-effect ODD systems. During the 21st century, numerous lipophilic and hydrophilic molecules have been incorporated into solid lipid nanobioparticles (SLNbp) for enhanced drug for extending, controlled release, better bioavailability [9, 12–16], and chemically stable protection extensive application [17, 18].

In recent years, polymers [19] have been used for development of advanced formulations to improve the bioavailability of drugs in systemic circulation. Furthermore, enhancing polymers, such as chitosans, thiomers or

carbomers [20–24], methacrylate polymer [25, 26], and herbal bio-enhancer [27–29] have been used to prepare advanced carriers for formulations. These protective vehicles were applied to avoid degradation in the GI tract, potentiate the absorption, and enhance bioavailability during delivery of drug through oral administration [5, 30–32]. In addition, statistical design applicability was limited experimental trails of investigational factors at different spaces provided effective optimized process formula of drug delivery. A number of factorial methodologies were applied to design, optimize, and develop SLN [33–37] systems successfully.

From literature, it is cleared that nowadays colloidal carrier has become as attention among pharmaceutical academic as well as research groups to developing novel predictable, prolonged periods of time, and improve bioavailability dosage forms. Accordingly, the main objective of our research was to prepare, characterize, and optimize isradipine (ISR; 15%–24% oral bioavailability and poor solubility) with enhancing agent (rutin) loaded nano-colloidal carrier *via* polymer (Eudragit L100)-coated, i.e., SLNbp using central composite design. Basically in this study, Eudragit (polymer grade EL100 meant for decline release in gastric acid fluid pH 1.2 due to its dissolution properties in intestinal fluid)-coated lipid carrier was develop and evaluate innovative nano bio-vehicle for oral delivery of ISR. The design helps to generate relationships between formulation-independent variables and levels by observed their dependent variables (responses) over elected entire experimental province for optimization. The optimization rationale is to find optimized nano-colloidal bio-carrier formula *via* polymeric coating to accomplish better stability, solubility, absorption, prolonged sustainable behavior (up to 40 h to reduce the dose), which might be an enhanced model drug (ISR) available through oral delivery.

Materials and Methods

A sample of ISR was procured from Orchid Chemicals and Pharmaceuticals Ltd., Tamil Nadu, India. Glycerol monostearate (GMS), soya lecithin, polysorbate 80 (PS 80), and organic solvents were purchased from Central Drug House Pvt. Ltd., India. The polymer Eudragit grade EL100 and rutin were obtained from Sigma Aldrich Chemicals Pvt. Ltd., India.

Preparation of nano-colloidal carrier via polymer coating

Initially, lipid-to-surfactant (GMS:soya lecithin) ratios were briefly melted (65–70 °C) in a rotary flask (Rotary Evaporator RV-10, IKA® India Private Ltd., Bangalore, India) and added equivalent ratio of ISR plus bio-enhancer organic (chloroform:methanol; 10 ml) solution as shown in *Table I*. The drug with rutin was entrapped into melted lipid–lecithin and solvent removed by evaporation process followed by the addition of polysorbate (PS 80; 1% w/w) aqueous solution to obtained dispersion. Concurrently, Eudragit (EL100) was dissolved in organic solvent (methanol) and transferred at above 5 °C of lipid melted temperature. Then, polymeric lipid dispersion was homogenized at 24,000 rpm for 15 min (T25, Digital Ultra-Turrax®, IKA India Pvt. Ltd., India) and sonicated immediately at 75% amplitude for 20 min (UP200S, Ultra-Probe Sonic, Hielscher-Ultrasound Technology, Germany) [8]. The

Table I Variables and levels in response surface methodology, i.e., central composite design

Independent	Variables Agent	Codes	Unit	−1 (low)	Levels 0 (central)	+1 (high)
Lipid-to-surfactant ratio	GMS:SL	A	% w/w	1.00 (5:5)	2.00 (10:5)	3.00 (15:5)
Eudragit L100	EL100	B	% w/w	0.75	1.00	1.25
Sonication time	ST	C	minutes	15.0	20.0	25.0
Fixed	Name	Codes	Unit		Quantity	
Drug	Isradipine	ISR	mg		2.5	
Bio-enhancer	Rutin	Ru	mg		2.5	
Co-surfactant	Polysorbate	PS80	% w/w		1.0	
Dependent	Abbreviation	Codes	Unit		Constraints	
Entrapment efficiency	EE	R1	%		Maximize	
Particle size	PS	R2	nm		Minimize	
Cumulative drug release	CDR	R3	%		Minimize	

GMS:SL = glyceryl monostearate:soya lecithin; EL100 = Eudragit L100

resulted nano-biosuspension converted into nano-bioparticles [26] by freeze-drying at −2 to 3 °C for 10 min and stored at 4 °C in anticipation of use.

Factorial design

Initially, preliminary trails were performed to find the significant factors appropriate ranges in which the quality level lie. After that a full factorial central composite design, i.e., response surface methodology (RSM) was applied to study the effect of selected factors responses by varying their levels (−1 and +1) to put quality level as central level [26]) individually or together in a limited number of experiments. The present statistical three factors at three-level systematic RSM design (Design Expert® version 10.0.2, Stat-Ease, Inc., Suite 480, Minneapolis, MN) were performed to exploit its appliance for oral delivery.

Methodology

A twenty formulation at low and high (−1 and +1) levels of quality central spaces [26] of independent (A, B, and C) factors and their responses (R1, R2, and R3) are mentioned in *Table I*. The second-order polynomial equation of design model is as follows: $R = b_0 + b_1A + b_2B + b_3C + b_{12}AB + b_{13}AC + b_{23}BC + b_{11}A^2 + b_{22}B^2 + b_{33}C^2$; where R is the dependent variable, b_0 is the intercept, b_1 to b_{33} regression coefficients of A, B, C, AB, BC, AC, A^2, B^2, and C^2 are computed from observed responses (R1, R2, and R_3) values. The numerous weight of factors A, B, and C was used to develop SLNbp (SLNbp 1–20) batches (their observed and predicted values are listed in *Table II*) to study the variables relative quantities effect in lieu of the SLNbp potential using quadratic design of RSM [34, 37, 38].

Characterization

Differential scanning calorimetry (DSC)

The DSC study was performed to find incompatibility and interaction between ISR, lipids, polymer, and formulations mixture *via* rutin. The thermal analysis (2.5 g) was performed on aluminum pan (used empty pan as reference) under nitrogen atmosphere at 10 °C/min and 40–300 °C temperature range.

Table II Central composite design variables responses

Batch	Variables								
	Independent			Dependent (responses)					
				Actual			Predicted		
Codes	A	B	C	R1	R2	R3	R1	R2	R3
SLNbp 1	−1	−1	−1	97.58	111.2	92.0	97.60	111.1	91.80
SLNbp 2	0	0	0	97.50	108.0	97.2	97.51	107.6	97.19
SLNbp 3	0	0	0	97.50	108.0	97.2	97.51	107.6	97.19
SLNbp 4	0	0	0	97.50	108.0	97.2	97.51	107.6	97.19
SLNbp 5	−1	−1	+1	96.21	110.4	93.8	96.23	110.3	93.72
SLNbp 6	0	0	−1	97.70	112.3	98.3	97.72	112.7	98.52
SLNbp 7	0	+1	0	97.71	106.9	95.7	97.67	107.3	95.64
SLNbp 8	+1	+1	−1	97.14	121.1	98.4	97.12	120.9	98.44
SLNbp 9	0	−1	0	97.20	105.9	94.8	97.23	106.3	95.04
SLNbp 10	−1	+1	−1	97.55	109.7	95.9	97.57	109.6	95.83
SLNbp 11	0	0	+1	97.50	105.3	97.5	97.47	105.7	97.46
SLNbp 12	+1	0	0	97.42	110.1	98.1	97.47	110.7	98.02
SLNbp 13	+1	−1	+1	97.10	104.4	97.2	97.08	104.2	97.23
SLNbp 14	0	0	0	97.50	108.0	97.5	97.51	107.6	97.19
SLNbp 15	−1	0	0	97.33	107.8	94.7	97.27	108.0	94.96
SLNbp 16	0	0	0	97.50	107.0	97.2	97.51	107.6	97.19
SLNbp 17	+1	+1	+1	97.95	107.9	94.4	97.96	107.7	94.48
SLNbp 18	+1	−1	−1	97.81	113.2	97.8	97.79	113.0	97.74
SLNbp 19	0	0	0	97.57	108.1	97.2	97.51	107.6	97.19
SLNbp 20	−1	+1	+1	97.74	104.4	94.2	97.77	104.3	94.22

A = lipid-to-surfactant ratio; B = Eudragit L100; C = sonication time; R1 = entrapment efficiency (%); R2 = particle size (nm); R3 = cumulative drug release (%)

Morphology evaluation by scanning and transmission electron microscopy (SEM and TEM)

The surface morphology of nanobioparticles was analyzed by SEM and TEM (Morgagni 268D, FEI Company, Hillsboro, OR, USA) exploited for the evaluation of shape and morphology. All formulations of particle size were analyzed using freshly centrifuged (LWs Combo V24T centrifuge, LW Scientific, Inc., India); the resulted suspension droplet was placed on a clean glass slide and dried in air. The diluted nano-biosuspension particle size was determined by dynamic light scattering (DLS) method, using a computerized inspection system (Zetasizer Nano ZS®, Malvern Instruments, Worcestershire, UK) in triplicate for the clarity.

Entrapment efficiency (%EE)

All batches (SLNbp 1–20) %EE was determined by dissolved and extracted with phosphate-buffered saline (PBS, pH 7.4; 5 ml) solution. A fixed volume of nano-dispersion (10 ml) was vortexed (2–3 min for dissolved free drug) followed by sonication (20 min; Ultra-sonic Bath, Multitech Instrument Co. Pvt. Ltd., New Delhi, India). Then, extracted ISR was mixed in PBS, and remaining solvents removed using vacuum evaporation (Rotary vacuum evaporator, Multitech Instrument Co. Pvt. Ltd., New Delhi, India). In addition, the obtained dispersion was micro-refrigerated and centrifuged (GRACE Centrifuge Instruments, Navi Mumbai, India) at 15,000 rpm for 40 min to eliminate the residue and collected the supernatant (1.5 ml). The absorbances of all batches-collected supernatants were recorded (Jasco V-630, UV-VIS Spectrophotometer, JASCO International Co. Ltd., Japan); system was calibrated and concentration range was found 2.5–15 µg/ml with regression equation $y = 0.157 \times -0.005$; $r^2 = 0.998$) against blank (PBS 7.4) as a reference at λ_{max} = 327 nm [38]. The EE in SLNbp and optimized solid lipid nanobioparticles (ONbp) was determined [26, 39] with the help of equation, i.e., $\%EE = C_{total} - C_{free}/C_{total} \times 100$, where C_{total} is the theoretical weight of drug and C_{free} is the free drug detected in supernatant.

Release studies

On basis of model drug ISR and rutin solubility, phosphate buffer (pH 7.4) media was selected to determine the formulations release in GI fluid using dialysis membrane method. The nano-biosuspension dispersion (equivalent to dose) was placed in gastro-fluid (pH 1.2 for 2 h because it would follow simulating from gastro to intestine during oral administration) before incorporated into dialysis bag (2.4 nm pore size) with a 12 kDa molecular weight cut-off membrane. Then, bag was immersed in a flask containing intestinal fluid medium (PBS, pH 7.4; 20 ml) at room temperature with constant stirring (control; pure drug suspension solution 50:50% PBS buffer and water) to maintain homogeneity. In addition, at known time intervals that are 0.5, 1, 2, 4, 8, 12, 16, 24, 32, and up to 40 h, after completion of each time, 5 ml aliquot was taken out and equally volume fresh buffer was added to made constant volume using side-arm inlet of receiver cell. The triplicate experiment samples were collected and assayed using UV spectrophotometer (λ_{max} = 327 nm) and calculate the release mean [6, 32].

Statistical analysis and optimization

A total of central composite design (20 runs) with triplicate center points from quality level were generated and prepared nanobioparticles observed responses were fitted to linear, cubic, quartic, and quadratic models simultaneously using statistical [34, 36] Design Expert® (10.0.2, Suite 480) software. The resultant investigational responses were compared with predicted values and all statistically model (*F* value) significant coefficients are included in the polynomial equations and design model response surface, actual versus predicted linear and residual plots were drawn. The constructed best-fitted quadratic model equations of responses (R1, R2, and R3) and their statistical R^2 values of significant coefficients were calculated by analysis of variance (ANOVA). After performed elected quadratic design experimentally; responses polynomial equations and model validation was done through numerical checkpoint prediction methodology to optimization.

Results and Discussion

During physicochemical evaluation, drug and all process variables have been exhibited and appeared sharp endothermic peaks indicated no chemical interaction, incompatibility, and instability [26]. The scanning electron micrographs were demonstrated *(Fig. 1)* discretely

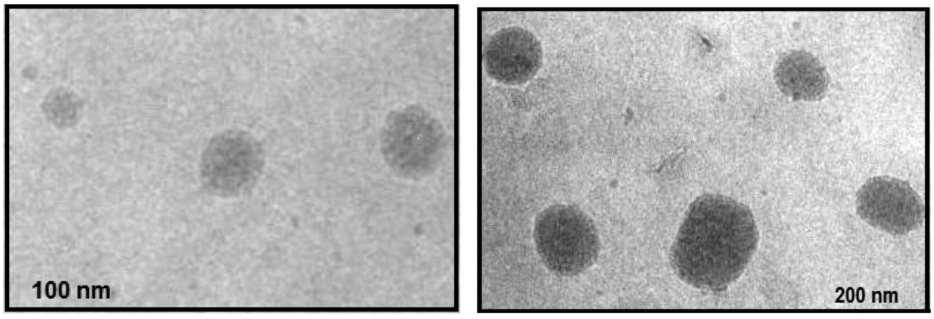


Fig. 1. Electronic imagery (SEM) of solid lipid nano-bioparticles (SLNbp)

deposited for SLNbp with smooth surfaces, which also revealed there was no roughness, irregularities, and wrinkles in shapes. Total maximum %EE obtained drug fraction into SLNbp ranging from 96.21% to 97.95%; substantially higher which is typically driven by oral gradients. Moreover, resultant higher efficiency was observed because of lipid carrier and its hydration core excellent capability of drug solubility along with outer polymeric layer provides stability. In addition, during evaluation of all batches (SLNbp 1–20), particle sizes and percentage cumulative drug release (%CDR) were found to be 104.4–121.1 and 92.0%–98.4% in nano-ranges *(Table II)*, respectively.

Responses equations analysis

The second-order quadratic equation was useful for identifying the relative impact of the factors by comparing the regression coefficients. Designed equations containing positive and negative values evidenced a directly and inversely interactive effect of variables (lipid-to-surfactant ratio, Eudragit L100 and sonication time; A, B, and C) individually and together on EE, particle size, and cumulative drug release (R1, R2, and R3, respectively).

Response 1: effect on EE

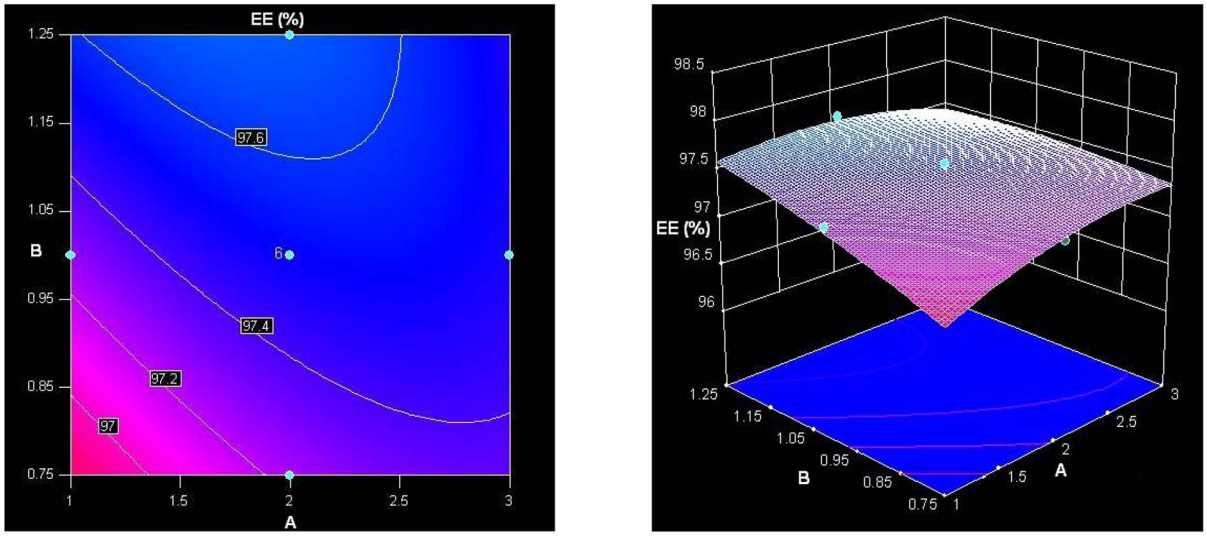
The response (R1) best-fitted quadratic model polynomial derived equation is as follows: $EE = 97.5 + 0.100A + 0.22B - 0.13C - 0.16AB + 0.16AC - 0.39BC - 0.14A^2 + 0.065B^2 - 0.080C^2$, where EE is the entrapment efficiency, A, B, and C are the lipid-to-surfactant ratios of the weight of polymer and sonication time, respectively. The model F value (156.36) implied; there is only a 0.01% chance that an F value could largely occur due to noise. The model ($p < 0.0001$; Prob. $> F < 0.0500$) values indicated A, B, C, AB, AC, BC, A^2, B^2, C^2 are significant terms and effect on EE. The lack of fit F value (3.29) implied the lack of fitness p value (0.1084 is greater than 0.1000) is indicative non-significance and predicted R^2 (0.9549) and adjusted R^2 (0.9866) have a reasonable agreement. An adequate signal ratio (58.438 greater than 4) value precision limit indicated that this model can be used to design levels. The investigational data also revealed that the efficiency was found to be less at low (−1) level as compared to central (0) level of factor A (lipid-to-surfactant ratio) along with factor B (polymer) had pronounced effect. This result evident that the efficiency and loading capacity was higher toward at central level of factor A and B lipids but at high (+1) level, it declined because of high lipid concentration made particles like mixed micelle (due to lipid association with surfactant particle), which made them as low-loading drug carrier [40], whereas factors A and C have direct relationship rather than B and C inversely proportional to EE. The combination of two factors at a time effect on SLNbp EE (R1) has also been shown by two- (contour) and three-dimensional (response surface) plots *(Figs 2a*, *3a*, and *4a)* simultaneously.

Response 2: effect on particle size (PS)

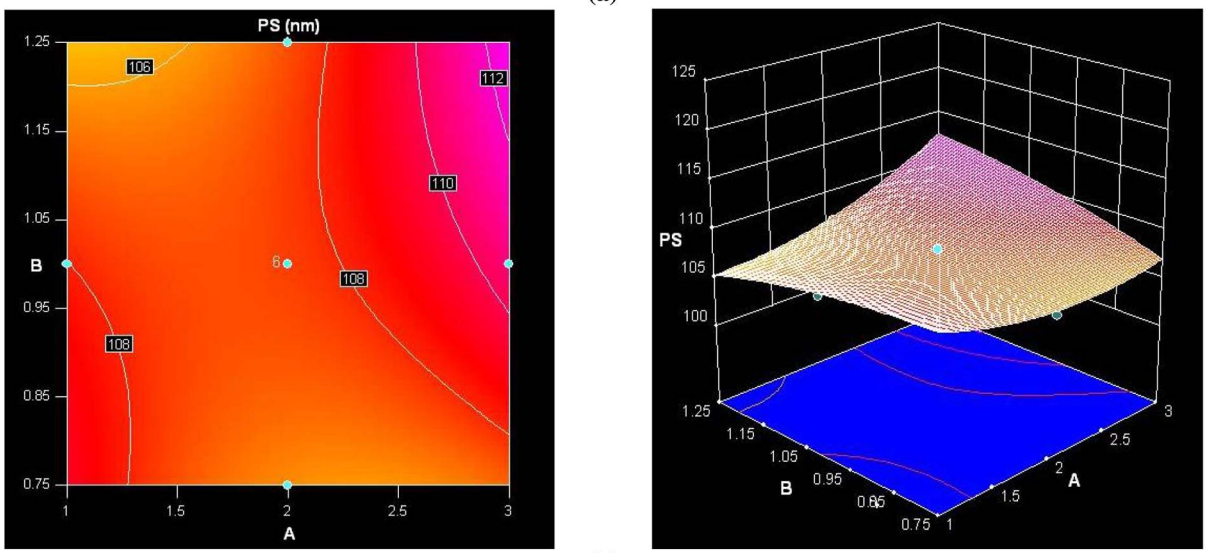
The following polynomial equation for particle size (R2) of SLNbp is prevailed from the model $PS = 107.58 + 1.32A + 0.49B - 3.51C + 2.36AB - 1.99AC - 1.11BC + 1.77A^2 - 0.78B^2 + 1.62C^2$; where PS is the particle size of the nanobioparticles. Overall quadratic model F value = 120.29 ($p < 0.0001$) significant, whereas the lack of fit F value = 1.77 ($p = 0.2734$; value greater than 0.1000) implies that fitness goodness is significant (lack of fitness is not significant, relative to the pure error). The p values less than 0.1000 indicated that model terms A, B, C, AB, AC, BC, A^2, B^2, and C^2 are significant and predicted R^2 (0.9703) have reasonable agreement with adjusted R^2 (0.9826) value. Moreover, signal-to-noise ratio is found to be in-limits as satisfactory with an adequate precision (observed ratio 47.986 is greater than 4) used to design the space. The independent factor C (sonication time [26]) had significant effect on the particle size; its negative coefficient in equation explore that at high level, size was decreased and vice versa. As well the factors A and B at low level with high level of C result smaller particle size. Instant this A and B positive coefficient showed direct influence on particle size which can affect the delivery of ISR; largest and smallest size at positive (+1) and negative (−1) level of factor A and B, respectively. The combined effects of factors AB, AC, and BC are demonstrated and shown in *Figs 2b*, *3b*, and *4b* correspondingly on particle size (R2).

Response 3: effect on drug release (%CDR)

The quadratic model purports the following polynomial equations and regression coefficients for response (R3) as follows: $CDR = 97.19 + 1.53A + 0.30B - 0.53C - 0.81AB - 0.59AC - 0.86BC - 0.70A^2 - 1.85B^2 - 0.80C^2$; where A, B, and C are independent factors *via* regression values. The polynomial equation coefficient positive and negative values are represented favorable and unfavorable effect of factor on the particular response. The quadratic model (F value = 205.46; $p < 0.0001$) and terms A, B, C, AB, AC, BC, A^2, B^2, and C^2 were found significant. The lack of fit (F value = 3.28; $p = 0.1090$; 10.90%) is not significant to the pure error. In this response terms, A and B had more pronounced effect on release rather than any others. The difference between predicted and adjusted R^2 (0.9705 and 0.9898, respectively) value is less than 0.2 proved that a reasonable agreement with each other. The adequate precision ratio (52.411) is greater than 4 indicates an adequate signal used to find



(a)



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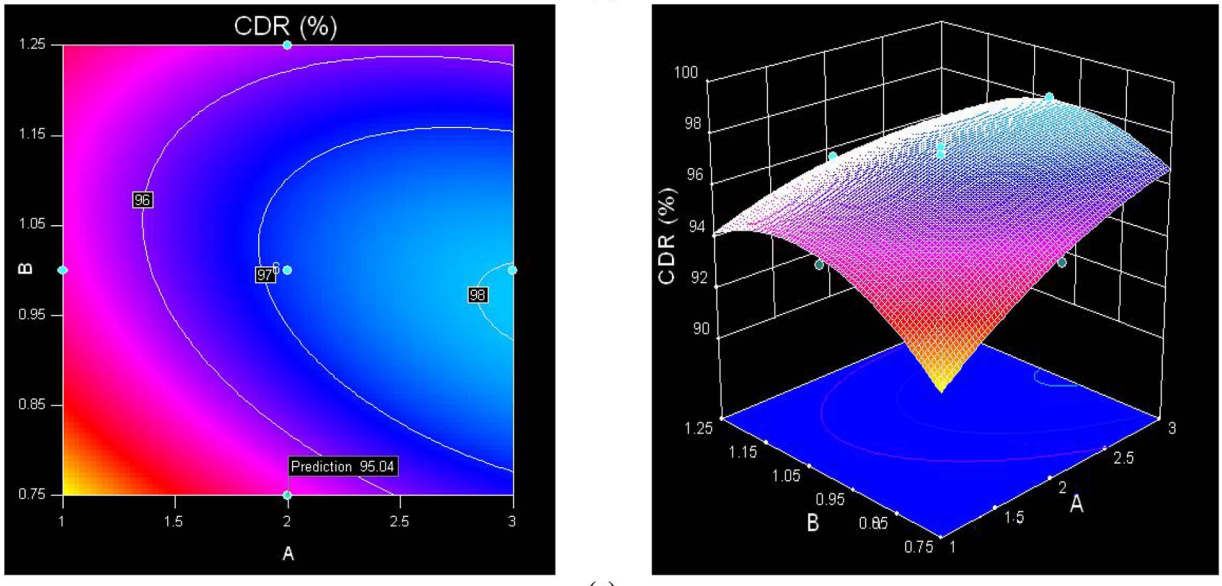

(c)

Fig. 2. Illustration of lipid-to-surfactant ratio (A) and polymer concentration (B) logical effect on (a) entrapment efficiency (%), (b) particle size (nm), and (c) cumulative drug release (%) by response surface (two- and three-dimensional) plots

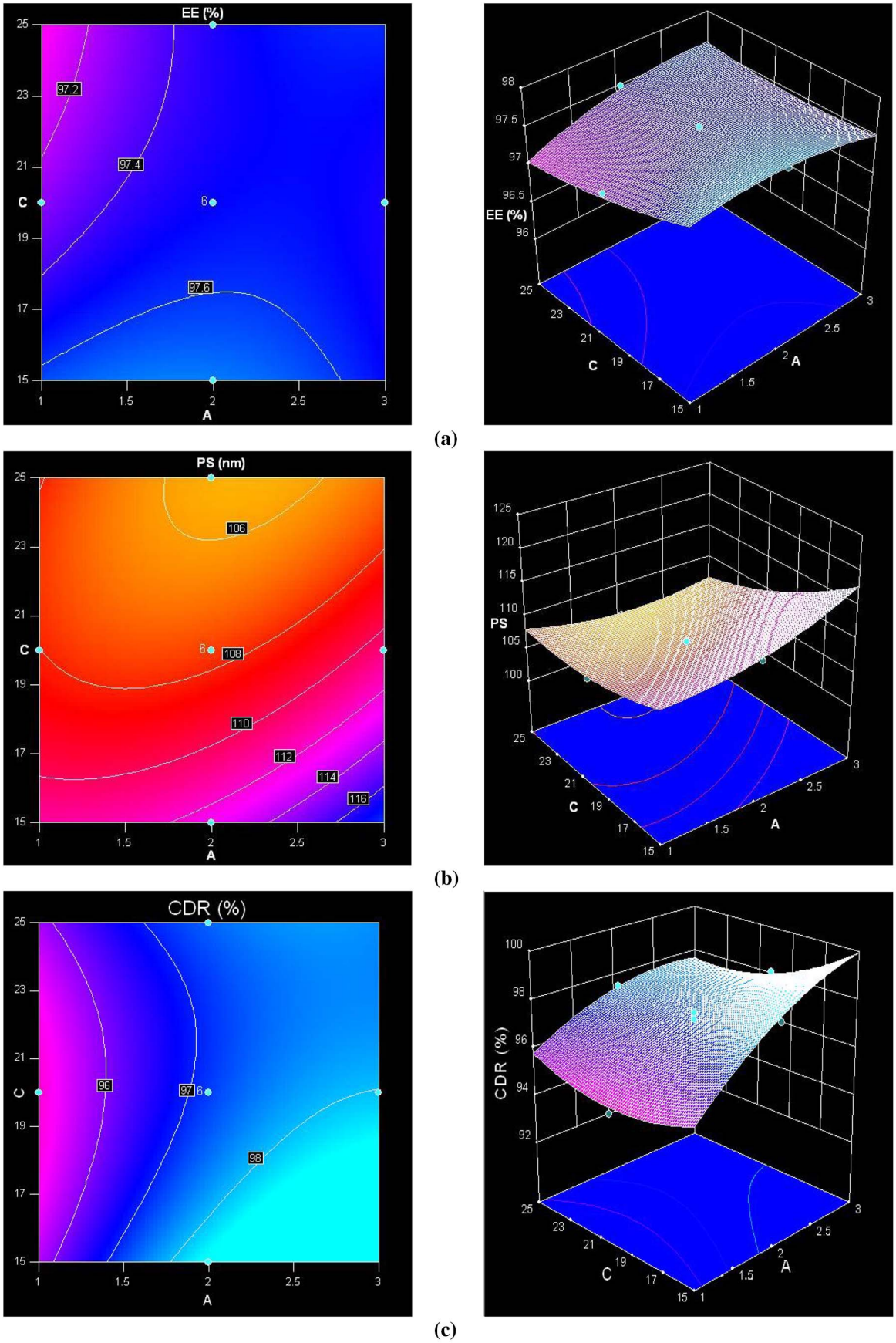

Fig. 3. Graphical image of lipid-to-surfactant ratio (A) and sonication time (C) interactive effect on (a) entrapment efficiency (%), (b) particle size (nm), and (c) cumulative drug release (%) by response surface (two- and three-dimensional) plots

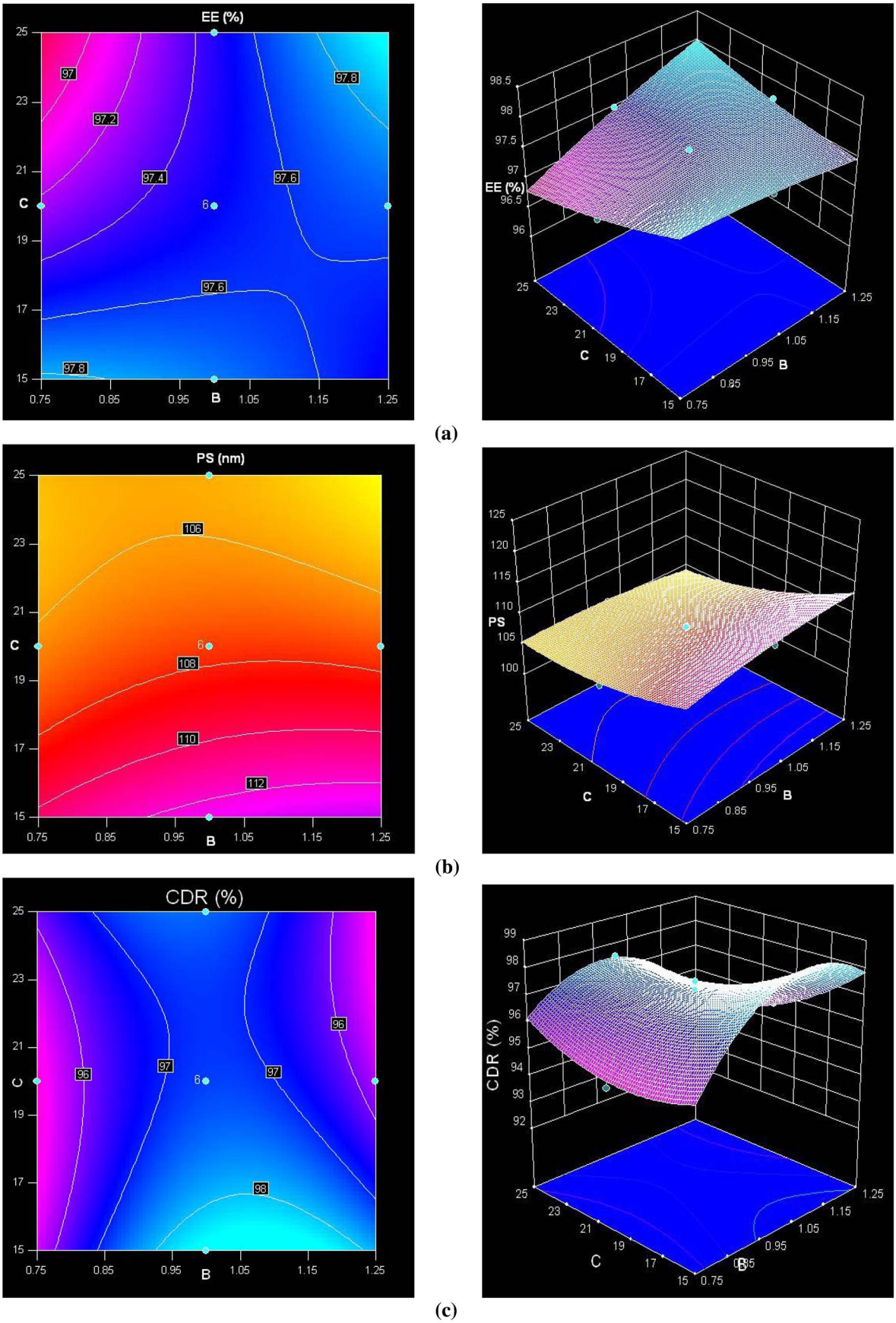

Fig. 4. Demonstration of polymer concentration (B) and sonication time (C) interactive effect on (a) entrapment efficiency (%), (b) particle size (nm), and (c) cumulative drug release (%) by response surface (two- and three-dimensional) plots

the design space. The contour and surface (two- and three-dimensional) plots were shown the interactive effects individually and together at a time on responses (*Figs 2c*, *3c*, and *4c* for R1, R2, and R3, respectively). The plots were also revealed that high value of AB has more positive effect as compared to factor AC and BC has negative effect at high (+1) level on drug release.

Optimization and validation

The best-fitted design model was found to be quadratic and model equations, R^2, standard deviation (*SD*), and coefficient of variance (%CV) values of responses (R1, R2, and R3) are depicted in *Table III*. The contour and response surface (two- and three-dimensional) plots showed factors (A, B, and C) individually and together (AB, BC, and AC) interaction effects *(Figs 2–4)* on the responses at same time. In addition, designed model good linear correlation found between actual versus predicted response along with residuals versus actual values, which have been illustrated in *Fig. 5*. The responses R^2 values of 0.9549 to 0.9929, 0.9703 to 0.9908, and 0.9905 to 0.9946 were in ranges for R1, R2, and R3, respectively and showed model excellent goodness of fit (lack of fitness is non-significant). Due to less magnitude along with significant R^2 values in the present investigation was proven the high-predictive aptitude during model optimization and validity. Therefore, numerical checkpoint prediction method was randomly selected optimum formulas (ONbp 1–10) based on the independent variables responses (R1, R2, and R3; 96%–98%, 104–121 nm, and 92.0%–98.5%, CDR ≤ 40 h) constraints. All optimum combinations (ONbp 1–10) were prepared to evaluate (%EE, particle size, and CDR vs. predicted; *Table IV*) experimentally and fitted to quadratic model to check percentage error to be within limits or not, further ensure validity and adequacy to find best optimized formula.

Optimized formulation

A numerical checkpoint strategy was predicted the best optimized nanobioparticles (ONbp 3); formulate and evaluate its morphology, size, EE, and drug release. The ONbp 3 transmission electron microscopic was justified and showed the morphology of polymeric-(outer surface)-lipid-(core)-nanobioparticles was spherical with uniform shape information (TEM clearly showed; lipid–lecithin core outer layer coated by Eudragit L100; dark in color illustrated in *Fig. 6*). Its process parameters (lipid-to-surfactant ratio 2.218, polymer concentration, 1.212 and sonication time, 23.79 (≃24 min) investigated responses have superior EE (97.85%) with good particle size (106.0 nm). From ONbp 3 formulation *in vitro* (CDR approx. 50% release in 7–8 h that mean drug exhibits higher stability in acidic condition) was 80.73% as compared to drug suspension (99.98%) and conventional SLN (≃95%) in 24 h (*Fig. 7* represented sustainable behavior). Moreover, formulation has prolonged release as a result of higher amount of drug encapsulating due to better solubility in lipid–lecithin core which also enhanced its absorption, polymeric coating prevented (only approx. 20%) drug release in gastric-fluid and enhancer (rutin; quercetin-3-O-rutinoside) inhibited [29, 30, 32, 41] enzymatic metabolism. To determine the release mechanism, data were applied to various kinetics models (zero, first, Higuchi and Hixson–Crowell; $R^2 = 0.869$, 0.975, and 0.973, respectively) and Korsmeyer–Peppas ($0.5 < n < 1.0$) higher R^2 value (0.983) indicated non-Fickian drug release mechanism. Overall, results were concluded that a polymeric (EL100)-coated SLNbp with bio-agent feature has great potential application as an ODD for lipophilic drug (ISR).

Table III Responses of quadratic model analysis and equations

Quadratic model	Responses	R^2 Actual	R^2 Adjusted	R^2 Predicted	*SD*	%CV
Response 1	EE (%)	0.9929	0.9866	0.9549	0.042	0.043
Response 2	PS (nm)	0.9908	0.9826	0.9703	0.49	0.45
Response 3	CDR (%)	0.9946	0.9898	0.9705	0.18	0.19
Polynomial equations		$R = b_0 + b_1A + b_2B + b_3C + b_{12}AB + b_{13}AC + b_{23}BC + b_{11}A^2 + b_{22}B^2 + b_{33}C^2$				
Response 1	EE (%)	$R1 = 97.50 + 0.10A + 0.22B - 0.13C - 0.16AB + 0.16AC - 0.39BC - 0.14A^2 + 0.065B^2 - 0.080C^2$				
Response 2	PS (nm)	$R2 = 107.58 + 1.32A + 0.49B - 3.51C + 2.36AB - 1.99AC - 1.11BC + 1.77A^2 - 0.78B^2 + 1.62C^2$				
Response 3	CDR (%)	$R3 = 97.19 + 1.53A + 0.30B - 0.53C - 0.81AB - 0.59AC - 0.86BC - 0.70A^2 - 1.85B^2 - 0.80C^2$				

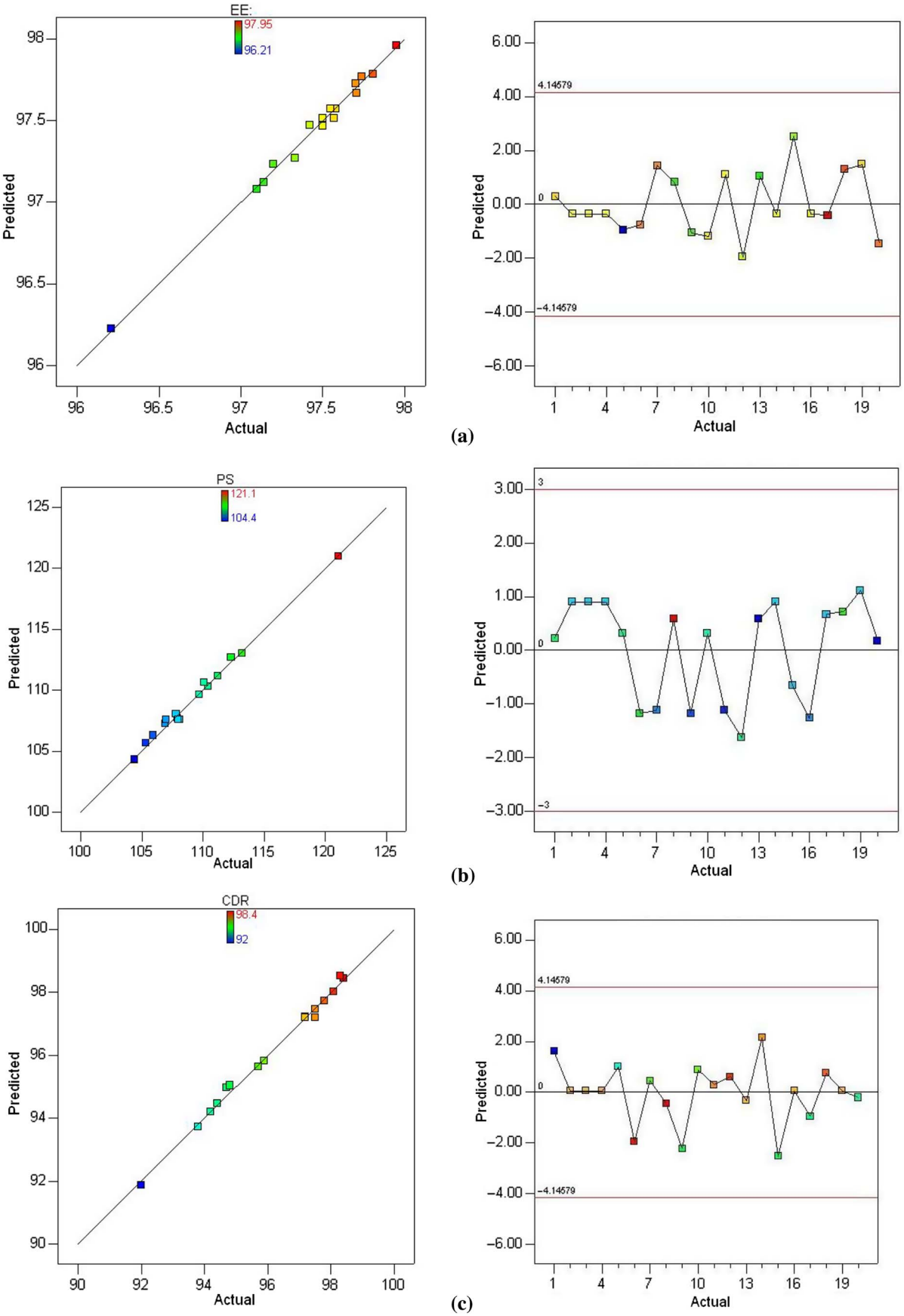

Fig. 5. Linear correlation and the corresponding residual plots of actual versus predicted values for (a) entrapment efficiency, (b) particle size, and (c) drug release

Table IV Numerical checkpoint optimum formulas responses (experimental and predicted) values

	Optimum factor compositions			Response variable values					
				Experimental			Predicted		
Optimized code	A	B	C	R1	R2	R3	R1	R2	R3
ONbp 1	1.046	1.201	24.53	97.75	105.5	94.98	97.64	105.1	94.82
ONbp 2	2.933	1.211	24.22	97.90	106.6	95.39	97.84	107.9	95.35
ONbp 3	**2.218**	**1.212**	**23.79**	**97.85**	**106.0**	**95.61**	**97.86**	**105.5**	**95.66**
ONbp 4	1.616	1.164	24.76	97.70	105.6	96.01	97.74	104.9	96.00
ONbp 5	2.321	1.131	24.23	97.72	106.5	96.70	97.77	105.8	96.70
ONbp 6	1.996	1.215	19.63	97.70	106.4	96.22	97.64	107.7	96.18
ONbp 7	2.547	1.075	23.90	97.65	107.0	97.17	97.67	106.4	97.23
ONbp 8	2.026	1.209	22.48	97.75	106.3	95.69	97.77	105.7	95.74
ONbp 9	2.633	1.199	24.49	97.84	107.2	95.76	97.89	106.4	95.76
ONbp 10	1.788	1.108	19.33	97.65	106.4	96.86	97.59	107.7	96.81

Best values are highlighted in bold

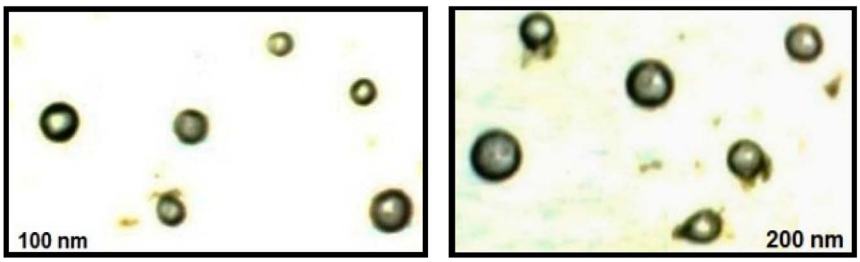

Fig. 6. TEM images of optimized solid lipid nanobioparticles (ONbp)

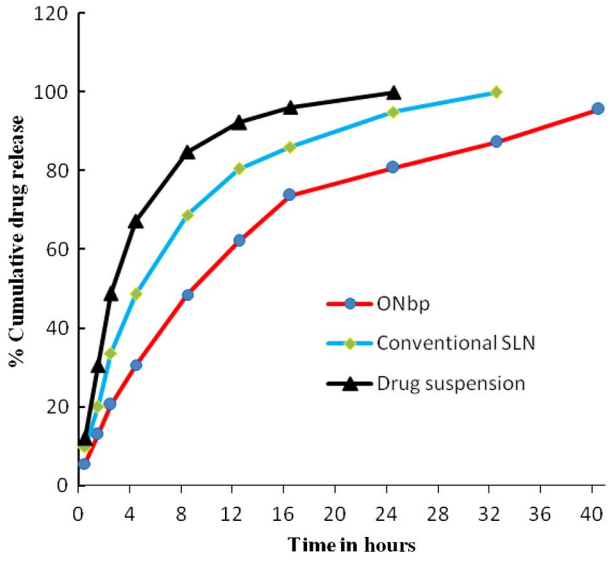

Fig. 7. ONbp 3 formulation drug release prolonged sustainable pattern as compared to drug suspension and SLN

Conclusion

The present research based on lipid nanoparticles optimized forms of polymeric lipid carrier (ONbp 3) of ISR (lipophilic drug with enhancer) was successfully prepared using RSM central composite design. The optimized formulation factor combinations quantitative effect at three levels have excellent linearity observed between actual versus predicted and of response *(Table IV)*. ONbp 3 was considered as the best due to small particle size (106 nm) provided a large surface area and achieved desired sustained effect which may get the desired bioavailability. Moreover, the sustained effect can help to maintain optimum release rate (up to 40 h); supported to a day continual dose reduction. This approach has been shown promising results; effective solubilization enhances absorption, prolonged sustainable release rate due to attain better oral availability of ISR by higher encapsulating (97.85%) into nano-colloidal carrier. Furthermore, there are no changes (appearances, color, uniformity, and separation) and drug content found 99.92 ± 0.08 during 3-month storage of ONbp 3 at 4 °C as per ICH [42] condition that indicated better stability. *In vitro* studies revealed significant sustainable (95.61%; *Fig. 7* showed in general 1.18- and 1.05-fold prolong healthier) release up to 40 h as compared to drug suspension and conventional SLN formulation, respectively. However, the results of optimized formulation (ONbp 3) to be considered as nano-therapeutic system which would be further subject

to pharmacodynamics and pharmacokinetics (and its adverse effect) activity, henceforth useful or not.

* * *

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Authors' contribution: The presented work was performed in collaboration with all authors. AK and HC participated in the study design. VK developed all the designed formulation and carried out their evaluation experimentally. VK, HC, and AK performed statistical analysis and interpretation of data along with manuscript preparation and editing. On the behalf of all authors, VK read and approved the final manuscript.

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