ORIGINAL ARTICLE

Solubility Enhancement of Resveratrol by Formulation of Solid Lipid Nanoparticles

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Abstract

Resveratrol is a stilbenoid, a type of natural phenol, and a phytoalexin produced by several plants such as grapes, blueberries, raspberries, mulberries, and peanuts with poor water solubility. The concept of reducing the particle size to nano dimensions was used to improve its solubility. Resveratrol-loaded solid lipid nanoparticles (SLNs) were developed using stearic acid as the lipid and poly vinyl alcohol (PVA) as the stabilizing agent. Resveratrol-loaded SLN were prepared using solvent evaporation technique and concentration of stearic acid and PVA were taken as the variables for optimization. Entrapment efficiency (EE) and yield of the SLN were taken as the dependent variables. All the responses observed for 13 formulations prepared were simultaneously fitted to linear, 2F1, quadratic, and cubic models using Design-Expert 10. It was observed that the best-fitted model was response surface, a quadratic model. SLN were characterized for yield, EE, particle size, zeta potential, and surface morphology. *In vitro* drug release studies were performed in phosphate buffer of pH 6.8 using dialysis bag diffusion technique. It was observed that in the case of SLN, the solubility was obtained approximately 3 times more than pure drug.

Key words: Nanocarrier, resveratrol, solid lipid nanoparticles, solubility enhancement

INTRODUCTION

Resveratrol, a natural polyphenolic component, has inspired considerable interest for its extensive physiological activities. However, the poor solubility of Resveratrol circumscribes its therapeutic applications. Resverastrol is a polyphenol found in grapes and red wines. Resveratrol is a drug, which has many pharmaceutical and pharmacological activities such as anticancer, anti-inflammatory, and antidiabetic.[1] It is an herbal extract derived from grapes and exhibits very few side effects with higher active potential. Resveratrol comes under biopharmaceutical classification system class II drug having low solubility and higher permeability.[2] When there is low solubility there are less chances for drug to reach at its specific part for its activity. Despite the beneficial therapeutic effects of Resveratrol, it has poor bioavailability, low water solubility, and is rapidly metabolized and excreted.[3] As a result, the free form of the drug is less suitable for drug delivery. Moreover, Resveratrol is also associated with dose-dependent side effects like nausea, vomiting, diarrhea, and liver dysfunction.[4] Among various techniques, solid lipid nanoparticles (SLNs) are one of the promising methods to improve solubility and dissolution characteristic of poorly water-soluble drug. Development of SLNs is a viable method to enhance bioavailability of poorly water-soluble drug, thus overcoming the limitation of previously used.[5] SLNs are exposed to aqueous media, the carrier dissolves and the drug releases as fine nanoparticles. The resulting enhanced surface area produces a higher dissolution rate and bioavailability of poorly water-soluble drug. Resveratrol has good absorption at small intestine and it is mainly absorbed through intestine.[6] In this study, an attempt to increase solubility of Resveratrol with the formulation of SLNs that will help to increase solubility and stability. Hence, the present work aims at formulating SLNs thereby increasing the solubility consequently decreasing the dose-dependent adverse effect/toxicity of the drug.

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MATERIALS AND METHODS

Resveratrol was obtained from Sami-Sabinsa Pvt. Ltd., Bengaluru. Tween 80, poly vinyl alcohol (PVA), methanol, stearic acid was procured from Sulab Laboratory, Vadodara.

Methodology

Resveratrol-loaded SLNs were formulated using the solvent evaporation method. Stearic acid was dissolved in 5 mL methanol. 20 mg of Resveratrol was dissolved in same solvent. The solution was sonicated using bath sonicator for 2 min. Then, solution was added to PVA solution (which contains 1% Tween 80) at room temperature with the help of a syringe and needle. This was further stirred for 2 h at 1500 rpm. Finally, the SLNs were separated using cooling centrifuge at 15000 rpm and at −10°C. The resultant SLNs which had settled below were collected.[7,8]

Solubility determination of drug in various solvents

Solubility of Resveratrol in various solvents such as distilled water, methanol, ethanol, phosphate buffer pH 6.8, and phosphate buffer pH 7.4 was determined using saturation solubility method. In this method, excess amount of drug was dissolved in 5 mL of each solvent separately in a test tube. These test tubes were kept in vertical shaker for 24 h to allow maximum drug to get dissolved. Then, resulting solution was centrifuged at 10000 rpm for 30 min and supernatant was taken (diluted, if necessary) and analyzed using a UV spectrophotometer.[9]

Calibration curves of drug in various solvents

10 mg of Resveratrol was dissolved in 10 mL of different media and then the volume was made up to 100 mL with distilled water. Appropriate aliquots from the stock solution of Resveratrol were transferred to 10 mL volume flasks and were diluted up to the mark with different media to prepare final drug concentrations of 20, 40, 60, 80, and 100 µg/mL. The absorption of all the prepared solutions was then measured at the absorbance maxima, 303 nm against the blank. The readings were recorded in triplicate. Mean value (n = 3) along with the standard deviation are recorded. The average values of absorption were plotted graphically against the concentrations and regression coefficient was obtained. Stability of the solution of Resveratrol in different media was ascertained by observing the changes in the absorbance of the solution at analytical wavelength over a period of 48 h at room temperature.[10] Procedure was followed for taking calibration curve in media like distilled water, methanol, ethanol, phosphate buffer pH 6.8, phosphate buffer pH 7.4, and 0.5, 1.0, and 1.5% w/v PVA solution.

Factor screening for SLNs formulation using response surface method design

The concentration of stearic acid and concentration of PVA were considered as the independent variables and their concentration was varied to fix the upper and the lower limits for the factorial design. Based on the limits selected, a 3^2 full factorial design was set up and various batches of Resveratrol-loaded SLNs were prepared accordingly. Tables 1 and 2 show upper and lower limits along with coded values.

Characterization of SLNs

Percentage yield (%)

The percentage yield of SLNs of various batches was calculated using the weight of final product after drying with respect to the initial total weight of the drug and polymer used for the preparation of nanoparticles. The percentage yield was calculated as per the equation as mentioned below.[11]

$$\text{Percentage Yield (\%)} = \frac{\text{Weight of dried SLN}}{\text{Weight of solid used (excipients + drug)}} \times 100$$

% drug entrapment efficiency (EE) separation of free drug

Analysis of Resveratrol from SLNs was done by separating free drug from the nanoparticles dispersion. The separation was done by centrifugation of nanoparticulate dispersion at 10,000 rpm for 30 min at −10°C (cooling centrifuge). Then, the nanoparticles and supernatant were separated.

Table 1: Response surface method design setup for optimization of SLNs formulation

Factor	Name	Name	Type	Minimum (–1)	Maximum(+1)
Independent factors					
X_1	Amount of stearic acid (mg)	-	Numeric	200	500
X_2	Concentration of PVA (%)	-	Numeric	0.5	1.5
Dependent factors					
Y_1	% Entrapment efficiency	% EE	Numeric response		
Y_2	% Yield	(%Y)	Numeric response		

SLNs: Solid lipid nanoparticles, PVA: Poly vinyl alcohol

Table 2: 3^2 Full factorial design layout of resveratrol-loaded SLNs

Sr. No	Batch code	Amount of stearic acid (X_1)	Concentration of PVA (X_2) (%)
1	RS01	200 mg (–1)	0.5 (–1)
2	RS02	200 mg (–1)	1 (0)
3	RS03	200 mg (–1)	1.5 (1)
4	RS04	350 mg (0)	0.5 (–1)
5	RS05	350 mg (0)	1 (0)
6	RS06	350 mg (0)	1.5 (1)
7	RS07	500 mg (1)	0.5 (–1)
8	RS08	500 mg (1)	1 (0)
9	RS09	500 mg (1)	1.5 (1)
10	RS10	350 mg (0)	1 (0)
11	RS11	350 mg (0)	1 (0)
12	RS12	350 mg (0)	1 (0)
13	RS13	350 mg (0)	1 (0)

SLNs: Solid lipid nanoparticles

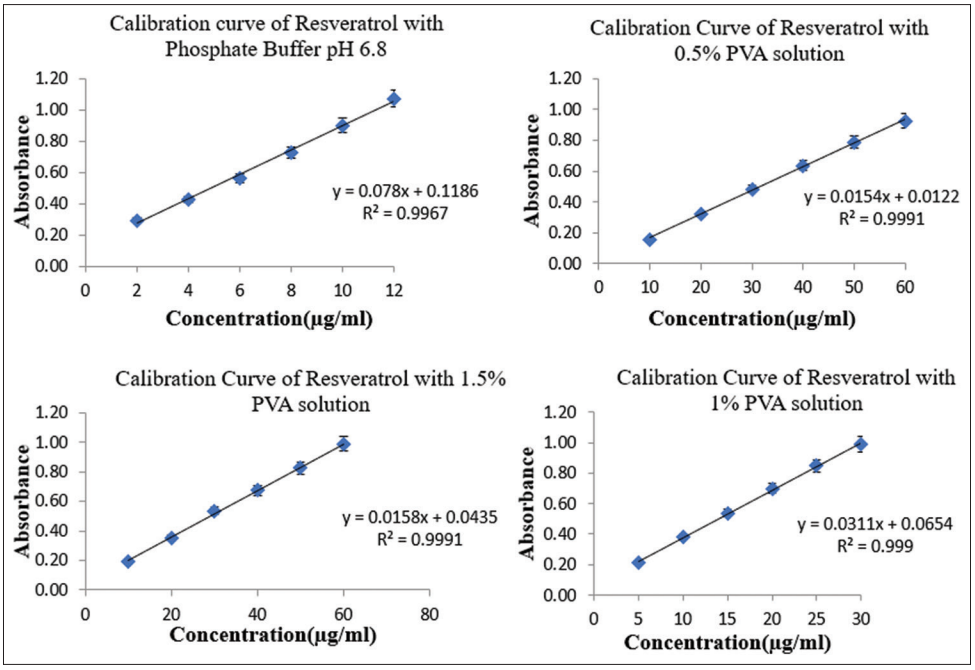

Figure 1: Standard calibration plot of resveratrolin various solvent

Analysis of Resveratrol from SLNs was done by indirect method. Centrifugation was followed by taking supernatant (diluted, if necessary) with PVA Solution (0.5, 1.0, and 1.5% w/w) and absorbance was taken against PVA Solution (0.5, 1.0, 1.5% w/v) as a blank on UV-Visible spectrophotometer (at 303 nm wavelength of absorption maxima).[11]

%EE was determined as per equation below:

$$\%EE = \frac{\text{Weight of Total drug} - \text{Weight of free drug}}{\text{Weight of Total drug}} \times 100$$

% drug content

The drug content in the SLNs was determined by dissolving 10 mg of dried SLN in 10 mL with distilled water. Absorbance of the resulting solution was then measured spectrophotometrically at 303 nm after filtration and appropriate dilution with distilled water. Drug content in the SLNs was determined as per equation below:

$$\%\text{Drug content} = \frac{\text{Practical drug content}}{\text{Weight of SLNs taken}} \times 100$$

In vitro drug release study

In vitro release studies were carried out using dialysis tubes with dialysis membrane having molecular weight of 12000–14000 Da. The prepared Resveratrol-loaded SLNs were redispersed in 5 mL of phosphate buffer pH 6.8 (strength 10 mg/mL) and subjected to dialysis by immersing the dialysis tube to the receptor compartment containing 50 mL of phosphate buffer pH 6.8. The medium in the receptor was agitated continuously using a magnetic stirrer and the temperature was maintained at 37 ± 0.5°C. 5 mL of sample from receptor compartment was taken at various intervals of time over a period of 24 h and each time, it was replaced with 5 mL of fresh phosphate buffer pH 6.8. The amount of drug released was determined spectrophotometrically at 303 nm.[11,12]

Particle size and zeta potential

The average diameter and zeta potential of an optimized formulation (RS05) were determined by photon correlation spectroscopy. It was carried out at the Department of Pharmacy, MSU, Vadodara. Nanosuspension in distilled water was added to the sample dispersion unit (deionized water) and stirred at 2000 rpm with magnet to reduce the inter-particulate aggregation. The samples were adequately diluted with deionized water and placed in an electrophoretic cell. The average particle size was measured after performing the experiment in triplicate.

Differential scanning calorimetry (DSC)

DSC is widely used in thermal analysis to monitor endothermic processes (melting, solid-solid phase transitions, and chemical degradation) as well as exothermic processes (crystallization and oxidative decomposition). It is extremely useful since it indicates the existence of possible drug excipients or excipient-excipient interactions in the formulation. Thermo grams of pure drug Resveratrol and excipients used in the formulation of SLN were obtained using a differential scanning calorimeter (Shimadzu). It was carried out at MSU, Vadodara. Samples were weighed directly in pierced DSC aluminum pan and scanned in the temperature range of 50–300°C under an atmosphere of dry nitrogen. Heating rate of 10°C/min was used and thermogram obtained was observed for interaction between drug and excipient.[13]

Scanning electron microscopy (SEM)

Surface morphology of optimized formulation (RS05) was obtained by scanning electron microscope. This study was carried out at EDRA, Vadodara. Electron gun produces a beam of electrons, which follows the vertical path through the microscope between electromagnetic fields and lenses, and X-rays are ejected from sample. Before examination, the samples were mounted onto metal stubs using a double-sided adhesive tape under vacuum. The scanning electron microscope was operated toward the sample due to electrons acceleration voltage of 25 kv.[14]

Stability studies

The optimized formulation of Resveratrol-loaded SLNs was placed in screw-capped glass container and stored at various ICH storage conditions, that is, 25°C ± 2°C, 45°C ± 2°C and 5°C ± 2°C for 45 days. The samples were analyzed for physical appearance, drug content, and *in vitro* drug release study at regular interval of 15 days.[14]

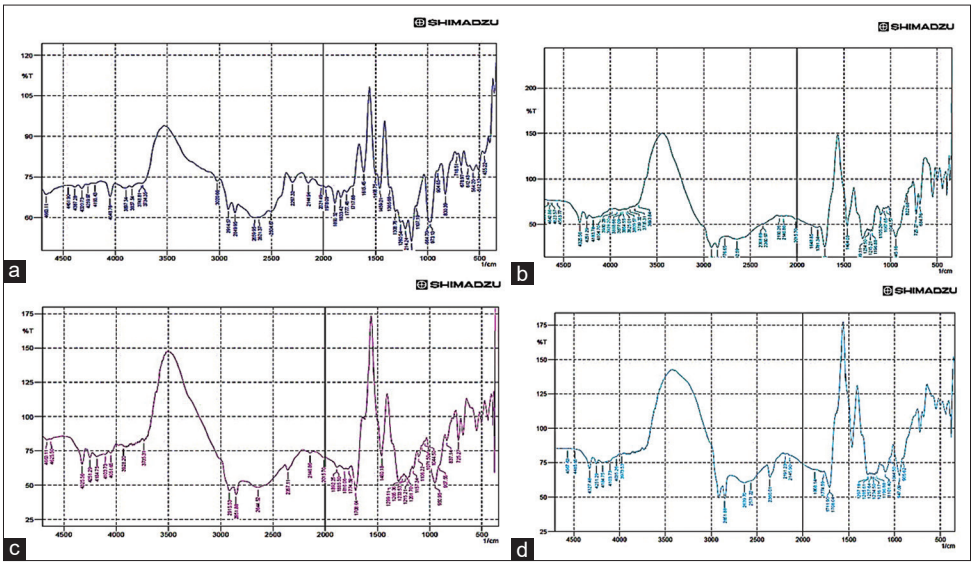

Figure 2: Fourier transform infrared (FT-IR) spectrum. (a) FTIR of resveratrol drug. (b) Stearic acid FTIR spectrum. (c) Resveratrol-stearic acid FTIR spectrum. (d) Resveratrol-loaded solid lipid nanoparticles FT-IR spectrum

RESULTS AND DISCUSSION

Calibration curve of resveratrol in various solvents

Calibration curve was plotted for Resveratrol in phosphate buffer pH 6.8, 0.5%, 1%, and 1.5% PVA solution using UV-Visible spectrophotometer at 303 nm. All the curves showed linearity and correlation coefficient above 0.99. All the calibration curves are displayed in Figure 1.

Compatibility study

Compatibility study of Resveratrol with various excipients was checked by FTIR spectrophotometric analysis using an FTIR instrument. The spectra of drug and excipients were taken which are displayed in Figure 2.

Optimization of SLNs by drug design

Factor screening using response surface method design results for Resveratrol-loaded SLNs formulation is highlighted in Table 3.

Response surface plots for Y_1 and Y_2

Response surface plots are very helpful in learning about both the main and interaction effects of the independent variables. This is shown in Figures 3 and 4.

Overlay plot

Overlay contour plot for formulation optimization is represented in Figure 5.

Characterization of optimized SLNs

In vitro drug release study of optimize SLNs and resveratrol

Comparative *in vitro* drug release profile of optimized formulation RS05 graphically represented in Figure 6.

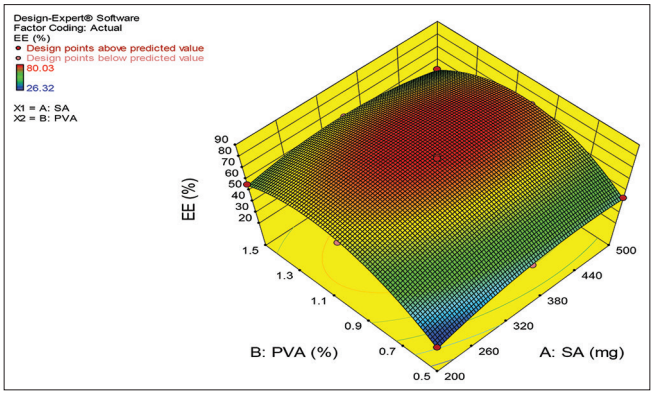

Figure 3: 3-D surface plot for solid lipid nanoparticles formulation for Y_1

Solubility comparison of optimize SLNS and resveratrol

The solubility study of the pure drug was found to be 0.20 ± 0.02 mg/mL whereas the solubility study of the optimized formulation (RS05) was found to be 0.56 ± 0.05 mg/mL and the SLNs.

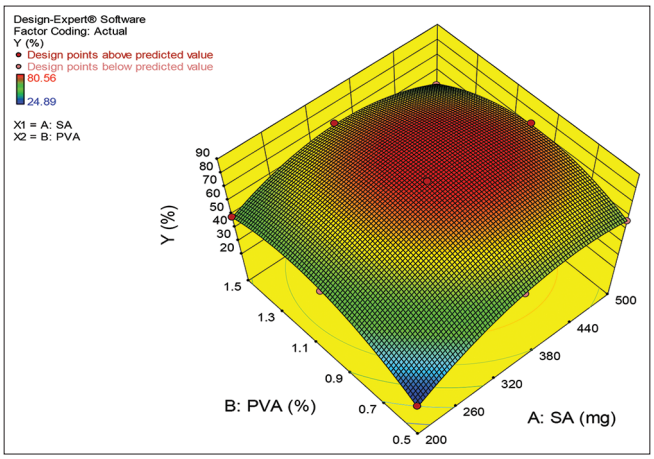

Figure 4: 3-D surface plot for solid lipid nanoparticles formulation for Y_2

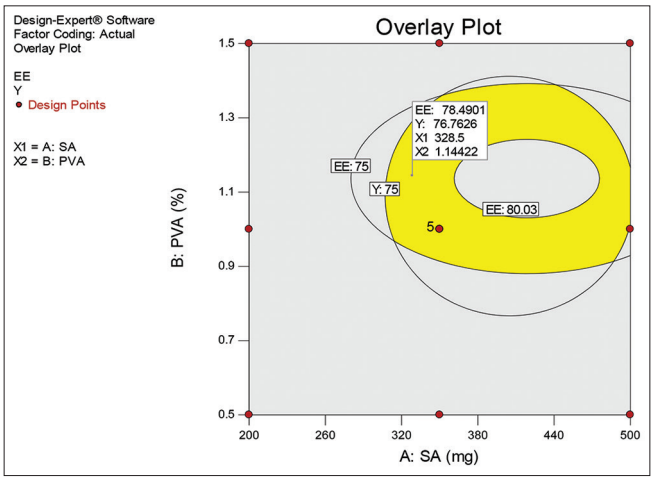

Figure 5: Overlay contour plot for solid lipid nanoparticles formulation optimization

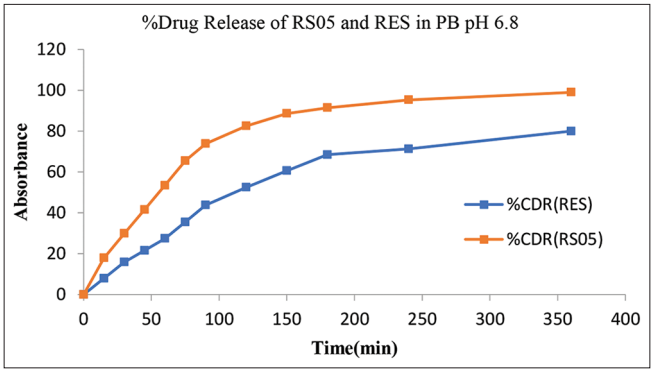

Figure 6: % CDR versus TIME of optimized batch RS05 and RES

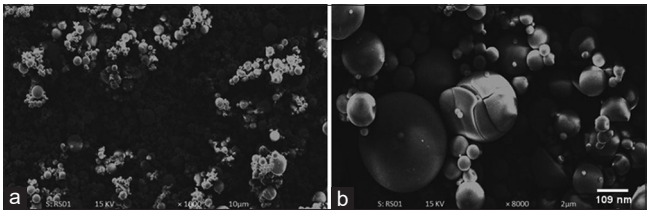

Figure 7: (a) *scanning electron microscopy* (SEM) image of optimized formulation at ×1000 magnification (b) SEM image of optimized formulation at ×8000 magnification

Evaluation of optimized SLNs formulation

Zeta potential and particle size distribution

Optimized formulation was evaluated for zeta potential and particle size distribution which was obtained as −14.5 mV and 594.5 nm, respectively.

SEM

The SEM study was performed to evaluate the particle shape, size, and distribution of Resveratrol-loaded SLNs.

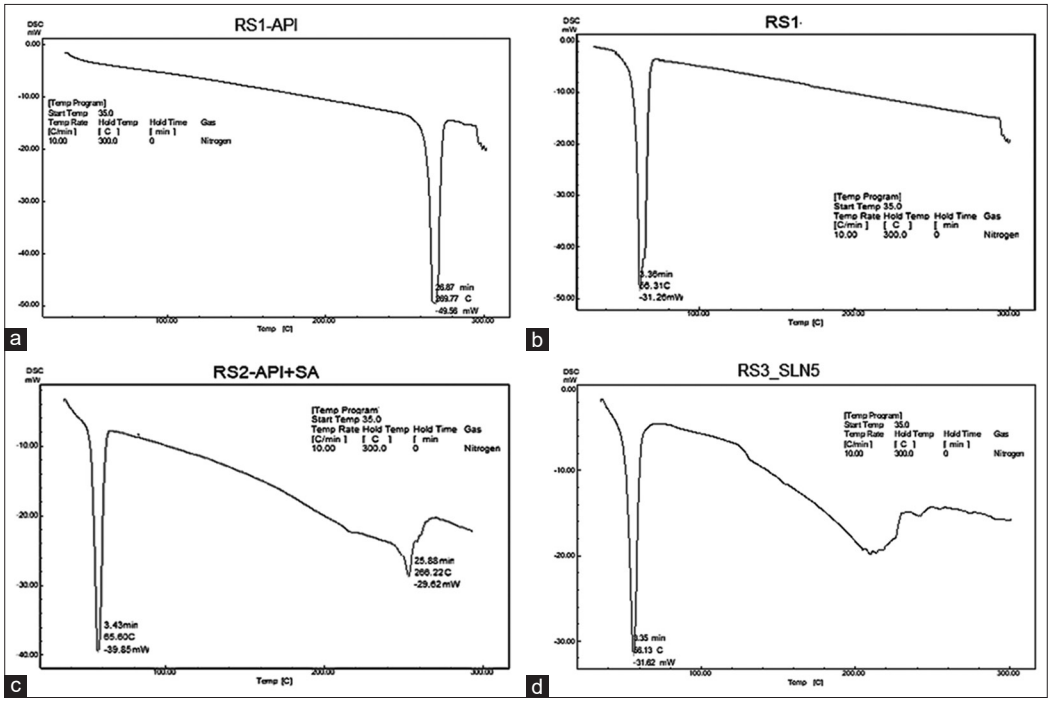

Figure 8: Differential scanning calorimetry (DSC) thermograms. (a) DSC of resveratrol. (b) DSC of stearic acid. (c) DSC of resveratrol + stearic acid. (d) DSC of solid lipid nanoparticles

Table 3: Factor screening using surface method design results for resveratrol-loaded SLNs formulation

Batch No	Amount of SA (X_1)	Concentration of PVA % (X_2)	% entrapment efficiency Y_1	% yield Y_2
RS01	200 mg (−1)	0.5 (−1)	26.32	49.35
RS02	200 mg (−1)	1 (0)	63.72	52.68
RS03	200 mg (−1)	1.5 (1)	56.49	43.96
RS04	350 mg (0)	0.5 (−1)	41.66	56.89
RS05	350 mg (0)	1 (0)	79.99	79.35
RS06	350 mg (0)	1.5 (1)	61.92	67.92
RS07	500 mg (1)	0.5 (−1)	44.29	57.09
RS08	500 mg (1)	1 (0)	71.84	72.65
RS09	500 mg (1)	1.5 (1)	69.43	71.56
RS10	350 mg (0)	1 (0)	78.56	80.56
RS11	350 mg (0)	1 (0)	80.03	79.09
RS12	350 mg (0)	1 (0)	78.67	78.63
RS13	350 mg (0)	1 (0)	77.98	79.92

SLNs: Solid lipid nanoparticles, PVA: Poly vinyl alcohol

[Figure 7a] displays SEM image of optimized formulation *at* ×1000 magnification whereas [Figure 7b] shows the SEM image of the optimized formulation at ×8000 magnification.

DSC study

The thermograms of the pure drug Resveratrol, stearic acid, Resveratrol + stearic acid, and SLNs formulation blend are shown in Figure 8.

Total drug content

The total drug used during the preparation of optimized batch of SLNs was 20 mg. Hence, using the formula the drug EE, the total drug content of Resveratrol-loaded SLNs was found to be 15.998 mg.

Stability study

The stability study was carried out for optimized formulation RS05. Stability studies were carried out at different temperatures for 45 days. The formulation RS05 was analyzed for visual appearance, drug content, and *in- vitro* release studies (at 6 h). The optimized formulation was subjected to stability studies at store SLNs for 45 days at different temperatures such as 5°C ± 2°C, 25°C ± 2°C, and 45°C ± 2°C. The formulation was evaluated for physical appearance, drug content, and *in vitro* drag release study at regular interval of 15 days. No major changes were observed in physical appearance, drug content, and *in-vitro* drug release profile when stored at room temperature. It indicates that the formulation was stable for longer period.

CONCLUSION

The present research work concludes that the solubility of BCS class II drugs can be improved if delivered through nanocarrier system. Resveratrol was selected for this project to improve the solubility by developing nanocarrier systems like SLNs. Resveratrol belongs to the BCS class II drug. An effort was done to formulate and evaluate the nanocarrier of Resveratrol for improving the solubility. Solvent evaporation method was used to develop the polymeric SLNs of the Resveratrol. The research work concludes that concentration of stearic acid (Lipid) and PVA (Stabilizing agent) showed to be a key factor to optimize the SLNs. The work demonstrated that drug entrapment and yield vary with the different concentrations of stearic acid and PVA in SLNs. The factorial design of optimization of formulation showed that SLNs of Resveratrol showed high drug entrapment (79.99 ± 0.50) at optimum concentration of 350 mg stearic acid and 1% of PVA. In SLNs, the solubility was obtained approximately 3 times more than pure drug which may be due to the effect of particle size reduction carried out during the solvent evaporation method. The research work further concludes that the dissolution rate of SLNs is faster when it compared with the plain drug. Thus, it can be concluded based on the result obtained that nanocarrier system of the selected drug can provide higher solubility than the pure drug.

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