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PREPARATION AND CHARACTERIZATION OF VALSARTAN LOADED SOLID LIPID NANOPARTICLES

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ABSTRACT
Nanotechnology is recognized as a landmark innovation and is considered "the sixth truly revolutionary technology introduced in the modern world". Nanotechnology has been widely explored in Anticancer Drug Therapy, Immunology, Antitubercular therapy, Cardiovascular Drug Delivery etc. While dealing with different categories of cardiovascular drugs the main obstacles in oral delivery is their low bioavailability. So here an attempt was made to formulate Solid lipid nanoparticles of Valsartan using high shear homogenization coupled with ultrasonication technique. Valsartan is a new potent, highly selective and orally active having low solubility, low bioavailability. The method was optimized by applying 3^2 level factorial design. The effects of composition of lipid materials and surfactant on particle size, drug entrapment efficiency and in vitro drug release behavior were investigated. The mean particles size, Polydispersity index (PDI) and entrapment efficiency of optimized formulation (V-10) was found to be 206.89 nm, 0.53, 80.46% respectively. The drug release study from the nanoformulation was studied in Phosphate buffer 6.8 for the optimized formulation (V-10). The results demonstrated that V-SLN formulation (V-10) showed biphasic behaviour with an initial burst release followed by a sustained release maximum up to 72-79% till 24 hours. The release curve was found to follow Korsmeyer Peppas model (R^2=0.98).

KEYWORDS: Valsartan, Solid Lipid Nanoparticles, Homogenization, PDI, Encapsulation efficiency.

INTRODUCTION
Nanotechnology is an integrative discipline, which represents a unique combination of classical natural, mathematical, computer and materials sciences, investigating and manipulating physical matter on the scale of nanometres. By the year 2000, nanotechnology was universally recognized as a landmark innovation, and named "the sixth truly revolutionary technology introduced in the modern world".[1] Over the last decade, nanotechnology has been extensively introduced into biomedical applications, including biological detection, drug delivery, diagnostic imaging and tissue engineering.[1] The nanometre scale is commonly indicated as 1-100nm, but nanoscience and nanotechnology often deal with objects larger than 100nm. 'Nano' means very small; but why is this special? There are various reasons why nanoscience and nanotechnology are so promising in material, engineering and related science.[2]

Lipid nanoparticles are at the forefront of the rapidly developing field of nanotechnology with several potential applications in drug delivery, clinical medicine and research. Lipid nanoparticles are basically the o/w emulsions in which oil part is replaced by solid lipid or mixture of solid lipid and liquid lipid. They were preliminary prepared as an alternative to other colloidal nanocarriers such as emulsion, liposomes and polymeric micro and nanoparticles. To combine their advantages. These lipids nanoparticles are known as solid lipid nanoparticles (SLNs) and nanostructured lipid carriers (NLCS) which are getting wide attention of formulators World Wide.[3,4]

Nanocarriers are colloidal systems having structures below a particles or droplets size of range 1-500nm. In 1990s researchers (Muller and coworkers and Gasco and coworkers) started the potential of nanoparticles made from solid lipids or solid lipid nanoparticles in the drug delivery. SLNS are composed of a biodegradable lipid matrix that is solid at body temperature and exhibit size

range in between 100 and 1000 nm.[5,6] SLNs have attracted increasing attention as an efficient and non-toxic alternative lipophilic colloidal drug carrier. SLNs are produced by replacing the oil of an o/w emulsion by a solid lipid or a blend of solid lipids.SLN are composed of up to 40% w/w solid lipid dispersed in an aqueous medium and stabilized with surfactant. They provide a controlled drug release and increase in chemical stability of the incorporated drugs. Moreover they are safe carrier which can be produced easily on large scale.[7,8,9]

High shear homogenization and ultrasound are dispersing techniques which were initially used for the production of solid lipid nanodispersions both methods are widespread and easy to handle.[10]

Valsartan is an Angiotensin receptor blocker (ARB) that selectively inhibits the binding of angiotensin II to AT1, which is found in many tissues such as vascular smooth muscle and the adrenal glands. This effectively inhibits the AT1-mediated vasoconstrictive and aldosterone-secreting effects of angiotensin II and results in a decrease in vascular resistance and blood pressure. Valsartan absorbed from upper GIT but has oral bioavailability 23% due to extensive hepatic first pass metabolism.[11,12] The water solubility of Valsartan is 0.0234mg/ml, Partition coefficient is 5.27 indicates that drug is highly lipophilic and can pass through lymphatic system. The pKa is 4.37 which indicates drug absorbed from acidic media.[13,14,15]

In this study an attempt is made to develop an efficient dosage form for delivery of Valsartan in the form of Solid lipid nanoparticles to improve bioavailability of Valsartan by avoiding its hepatic metabolism. Also the aim of this work was to study the effect of various lipids (Compritol ATO 888 & Glyceryl Monostearate (GMS) and surfactant like Poloxamer 188 in different concentrations on entrapment efficiency (EE), particle size of the solid lipid nanoparticles. To test the hypothesis to determine whether Compritol ATO 888 and GMS lipid are suitable for preparation of Valsartan loaded solid lipid nanoparticles by High shear Homogenization method coupled with Ultrasonication.

MATERIALS AND METHODS

Materials: Valsartan was obtained as gift sample from Hetero Chemicals, Hyderabad. Compritol ATO 888 and Poloxamer 188 was provided as gift sample from IPCA Laboratories, Silvasa, Dadra and Nagar Haveli. GMS was obtained as gift sample from BASF, Turbhe, Navi Mumbai. All chemicals were analytical grades and used as received.

Preparation of Valsartan loaded Solid lipid Nanoparticles: The preparation of SLNs dispersion was based on the principle of HSH coupled with ultrasound method. Lipid (Compritol 888 ATO and GMS) was melted at about 80^0C (10^0C above the melting point of lipid) and drug was added to obtain clear melting solution. An aqueous phase was prepared by dissolving surfactant (Poloxamer 188) in distilled water and heated to same temperature of oil phase. Hot aqueous phase was added to the oily phase and homogenization was carried out (8000 rpm and temperature 80^0C) for 15 min by using Ultra-turrax. Further oil in water emulsion so obtained was ultrasonicated using ultrasonicator for 10 min. Valsartan- SLNs were obtained by allowing hot nanoemulsion to cool 4-8^0C under magnetic stirring for 10-15 min. After cooling it was stored in refrigerator.

Experimental Design

Study design

Optimization design type: 3^2 level factorial design.

Software: Design Expert Version 10.0.0.3.

In the present study an attempt was made to design and optimize Valsartan loaded SLNs, by High shear homogenization (HSH) coupled Ultrasonication method. Based on the results obtained in preliminary experiments, concentration of lipid [X_1] and concentration of surfactant [X_2] were found to be the major variables in determining the particle size (PS) and the entrapment efficiency (% EE). So, these variables were selected to obtain an optimized formula for minimum PS and maximum % EE using 3^2 factorial design. The effect of these independent variables was investigated on two dependent variables, namely particle size [Y_1] and % entrapment efficiency [Y_2].

The operating conditions i.e. the rpm and time of ultra-turrex in the primary emulsions and the time for ultrasonication were adjusted based on results obtained in preliminary study and were latter kept constant during runs of optimization batches. The emulsification of the ingredients by Ultra- turrax was performed at 8000 rpm for 15 min, followed by ultrasonication using ultrasonicator (Citizen) for 10 min.

Table. 1: Coded and actual values of the formulation variables of factorial design.

Factor	Levels		
	Low (-1)	Medium (0)	High (1)
Independent Variables			
Lipid conc in % [X1]	2	3	4
Surfactant conc in % [X2]	0.5	1	1.5
Dependent Variables	**Goal**		
Particle size [Y1]	Minimum		
EE [Y2]	Maximum		

Formulation optimization: The experimental run generated by Design-Expert software version 10.0.0.3 were formulated with 0.5%w/w of Valsartan and subjected to evaluation studies for responses (table 7.2). Coded values and actual values of two independent variables, amount of lipid (X1) and surfactant concentration (X2) and dependent variables with their goals are represented in table 1 and 2.

Table 2: 3^2 level factorial design

Run	Conc. of Drug (mg)	Conc. Of Compritol ATO 888 (gm)	Conc. of Poloxomer 188 (gm)	Conc. of glyceryl monostearate (gm)
V-1	80	2	0.5	1
V-2	80	2	1	1
V-3	80	2	1.5	1
V-4	80	3	0.5	1
V-5	80	3	1	1
V-6	80	3	1.5	1
V-7	80	4	0.5	1
V-8	80	4	1	1
V-9	80	4	1.5	1

Evaluation parameters

ATR spectrophotometric study

ATR spectra of pure Valsartan and its physical mixture with lipid and surfactant were recorded on Shimadzu ATR spectrophotometer. The instrument was operated under dry air purge and the scans were collected with resolution of 4cm^{-1} over the region 4000-400 cm^{-1}. The scans were evaluated for presence of principle peaks of drug, shifting and masking of drug peaks and appearance of new peaks due to polymer interaction.

Screening of Solid Lipids

Solubility of the drug in a lipid is a key factor to achieve high entrapment of the drug into the lipid matrix. Therefore, solubility of Valsartan in various lipids was determined in order to determine the lipid having maximum potential to solubilize the Valsartan.

For studying the solubility in solid lipids, accurately weighed 10mg of the Valsartan was taken in a test tube, the solid lipid was added in increments and the test tubes was heated in a controlled temperature water bath kept at 10^0C above the melting point of the respective lipid. The test tube was observed for a drug residue. The amount of lipid required to completely solubilize the Valsartan in the molten state was estimated.

SEM

Scanning Electron microscopy (SEM) was performed to get morphological image of size and shape of nanoparticles formed. The SEM studies were carried out by Gold ion coating of sample for 5 min followed by image capturing.[18]

Make/Model: SEM Jeol JSM-6510

Particle size analysis and Polydispersity index

The nanoparticles formulation was suitably diluted with deionised water till an optimum intensity was achieved. The particle size was analysed using Delsa^{TM} nanosizer (Beckman Coulter).[16, 17]

Zeta Potential analysis

The V-SLN formulation was suitably diluted with deionised water till an optimum intensity was achieved. The zeta potential was analysed using Malvern zetasizer.[17]

Entrapment efficiency

The entrapment efficiency of V-SLN was determined by separation of nanoparticles from aqueous medium (supernatant) by centrifugation and absorbance of free Valsartan was determined by UV ay 250 nm. The amount of Valsartan was calculated using the standard curve. [16]

$$\%\text{Entrapment efficiency} = \frac{initial\ weight - weight\ of\ free\ drug}{\text{weight of initial drug}} \times 100$$

***In vitro* release study**

Plain drug: Valsartan

The drug release of Valsartan was studied using dialysis bag diffusion technique. The release of Valsartan was studied across the dialysis membrane-50 (Himedia) which was used as synthetic barrier and hydrated with receiver medium before experiments. The drug equivalent to 8mg was transferred to a dialysis bag and sealed. Samples (2ml) were collected at fixed intervals for up to 60min (10, 20, 30, 40, 50 and 60min) and replaced with solvent (2ml). The drug concentrations in the samples were determined by UV Spectrophotometry.

Drug release from V-SLN

The *in vitro* release of Valsartan from different SLN dispersions was determined using the dialysis bag diffusion technique. An accurately weighed amount of

Valsartan-loaded SLN dispersions containing the drug equivalent to 8mg was transferred to a dialysis bag and sealed. The sealed bag was then suspended in a beaker containing 250 ml of Phosphate buffer 6.8 and stirred at a constant speed of 50 rpm at 37°C ± 0.5°C. Aliquots were withdrawn at predetermined intervals from the receptor compartment up to 24 hours and the same was replaced with fresh buffer. Then the drug content was determined spectrophotometrically by measuring the absorbance at 250 nm using the respective receptor medium as a blank, to calculate the amount of drug release from nanoparticles.[16,19]

RESULTS AND DISCUSSION

Initially Compritol ATO 888 was examined for formulation of nanoparticles and it was found that it has minimum encapsulation efficiency and larger particle size result in lower surface area. So in the present investigation GMS was combined with Compritol ATO 888.

Optimization results

Results of factorial design: Two responses viz. response-I (particle size) and response-II (%EE) were recorded and processed by design expert software as shown in table 3.

Table. 3: Formulation of factorial design and their responses.

Run	Conc. of Drug (mg)	Conc. Of Compritol ATO 888 (g)	Conc. of Poloxamer 188 (g)	Conc. of glyceryl Monostearate (g)	Particle size(nm)	EE%
V-1	80	2	0.5	1	178	54.05
V-2	80	2	1	1	167.05	59.96
V-3	80	2	1.5	1	162.92	60.97
V-4	80	3	0.5	1	193.23	66.03
V-5	80	3	1	1	189.95	70.49
V-6	80	3	1.5	1	182	72.62
V-7	80	4	0.5	1	229	75.67
V-8	80	4	1	1	215.01	78.97
V-9	80	4	1.5	1	203.36	80.52

Table. 4: Selection of goal for optimization.

Response	Name	Minimum	Maximum	Goal
Y1	Particle size (nm)	162.92	229	Minimum
Y2	% entrapment efficiency	54.05	80.52	Maximum

ATR spectrophotometric study

The ATR spectrum of pure drug and its physical mixture were shown in fig. 1 and 2. The characteristic peaks of Valsartan were observed in the range from 763.81 cm^{-1} C-H aromatic stretching, 1728.22 cm^{-1} C=O stretching in carbonyl, 1273.02 cm^{-1} C-N amine stretching, 3657.04 cm^{-1} O-H stretching and 1465.90 cm^{-1} C=C aromatic stretching. These peaks indicated that there was no major change in the position of peaks obtained in pure API and its physical mixture. Thus it was proved that there was no chemical interaction between drug and excipient.

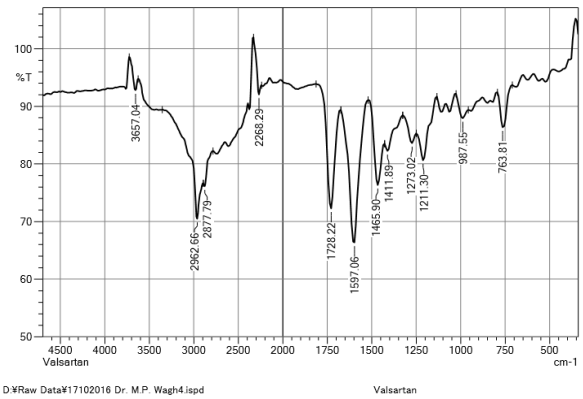


Fig 1: ATR spectrum of Valsartan.

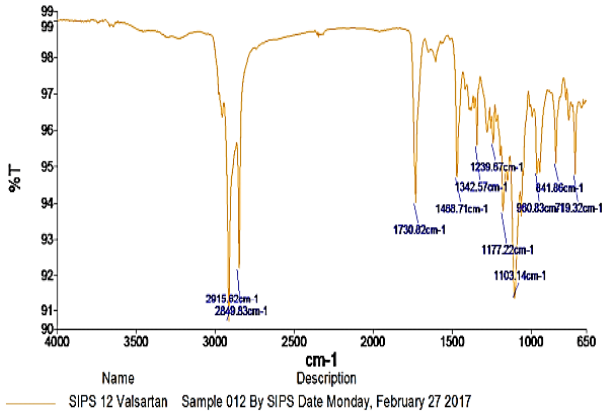


Fig 2: ATR spectra of physical mixture of drug with lipids and surfactant.

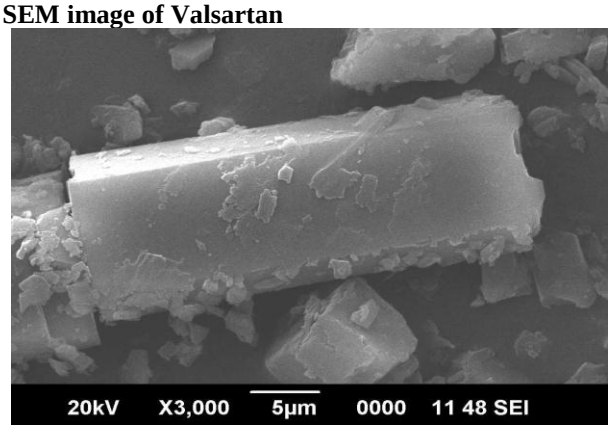
Compared to original Valsartan the broad band around 2915.82 cm^{-1} attributed to C-H stretching vibration was found slightly shifted spectra. The sharp peak at 1730.82 cm^{-1} attributed to –C=O of acid remain unaffected.

Solubility of drug in lipids

Table 5: Lipids required for drug solubilisation

Lipid	Amount required to solubilised drug
Compritol 888 ATO	110.91mg
Glyceryl Monostearate	463.7mg

SEM image of Valsartan



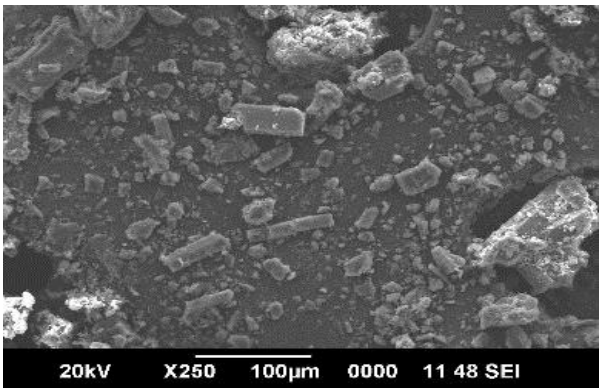


Fig. 3: SEM image of Valsartan.

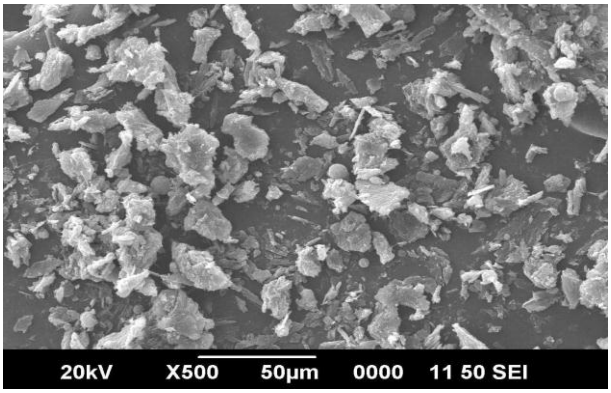


Fig. 4: SEM image of Valsartan loaded SLNs (V-10).

The SEM micrograph of Valsartan loaded SLNs is presented above in fig. The nanoparticles with little rough surface. As compared to the plain drug SEM is different. The particles appear to be irregular in shape and show diversity in particle size.

Particle size distribution curve of V-SLNs

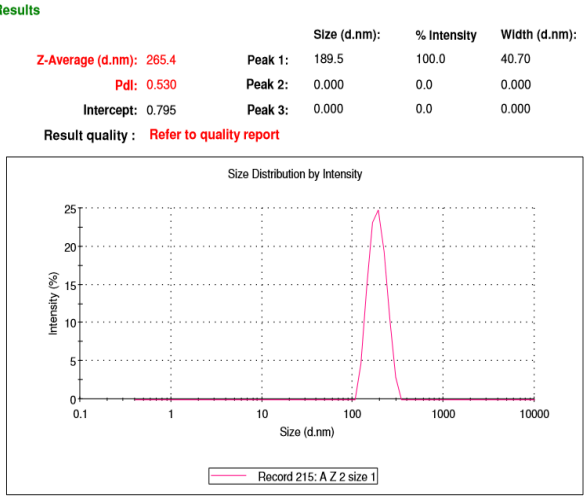


Fig. 5: Particle size distribution curve of V-SLNs (V-10).

The particle size of all batches V1 to V9 was found in the range 162.92 to 229.00 nm. The average particle size of optimized batch (V-10) found to be 265.4 nm.

PDI value of V-10 formulation **0.530**. PDI value below **0.5** for SLNs shows good homogeneity.

Contour and response surface plots

Contour plots and response surface plots (RSP) were useful in the study of the effect of two factors on a response at a time.

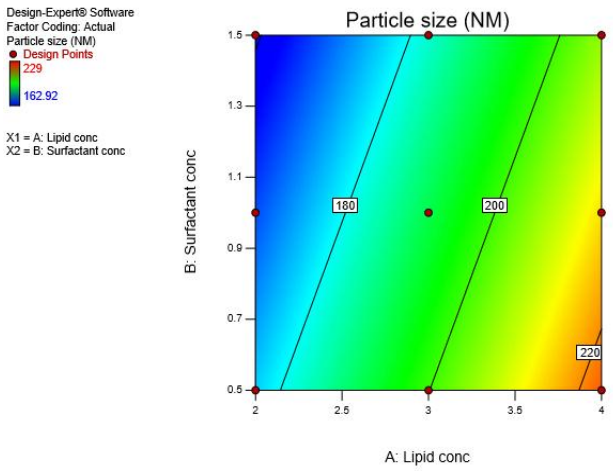


Fig. 6: Contour plot showing the effect of lipid conc. and surfactant concentration on Particle size.

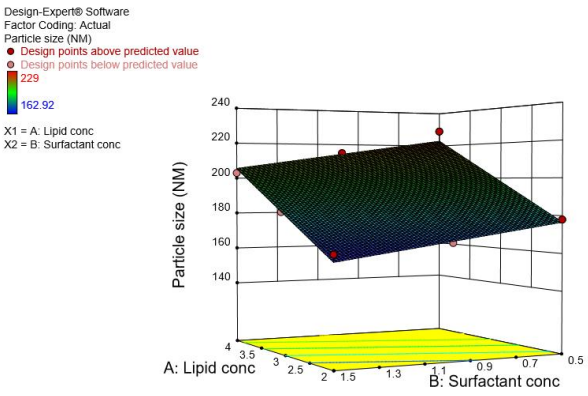


Fig. 7: Response surface plot showing the effect of lipid concentration and surfactant conc. on particle size.

Increased amount of lipid caused an increase in particle size (fig 6 and fig 7), due to the tendency of the lipids to coalescence at a higher concentration. On increasing the concentration of Poloxamer 188, the particle size was decreased. This might be due to the surfactant –induced reduction in surface tension between aqueous surfactant phase and lipid phase. In addition surfactant helps to stabilize the newly generated surfaces and prevents particle aggregation.

Entrapment Efficiency

Contour and response surface plots

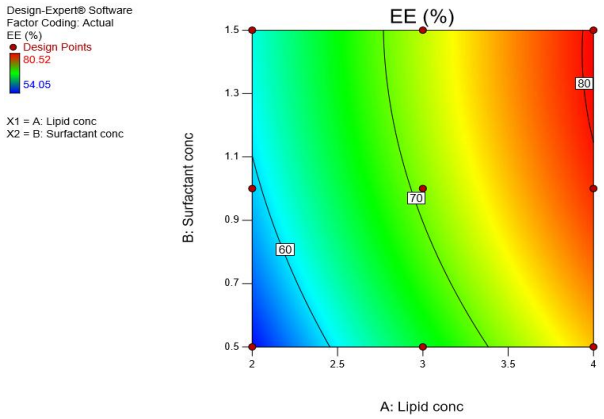


Fig 8: Contour plot showing the effect of lipid and surfactant conc on % EE.

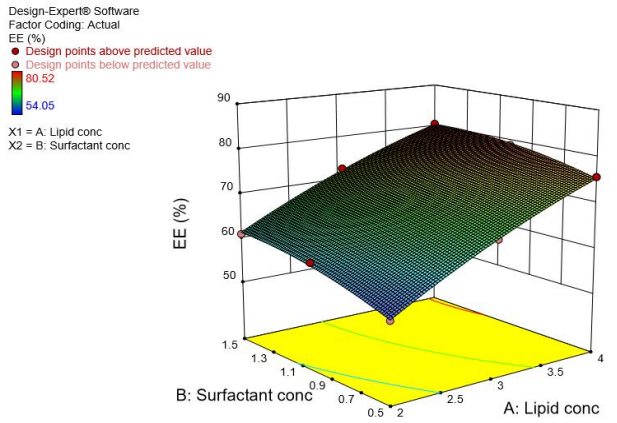


Fig. 9: Response surface plot showing the effect of lipid and surfactant conc on % EE.

The contour plot (fig 8) and response surface plot (fig 9) shows that as concentration of lipid increases, % EE increases. This may be due to the reason that with increase in concentration of lipid more amount of drug is entrapped hence less drug remains free in dispersion which in turn increases % EE and also due to use of two lipid they form more complex structure between two lipid layer and crystal imperfection hence more drug get loaded in lipid while as the concentration of surfactant increases, the % EE also increases as surfactant act as a stearic stabilizer.

Table. 6: Composition of batch generated by the software and its predicted response.

Predicted Response values				
Number	**Lipid %**	**Surfactant conc %**	**Particle size**	**% EE**
1	4	1.425	206.89	80.46

Based on the predicted response, experiment batch was performed and results as shown in table 6.
The final experimental batch coded as V-10

Table. 7: Experimental batch and its response.

Experimental response				
Number	**Lipid %**	**Surfactant conc. %**	**Particle size**	**% EE**
V-10	4	1.425	189.5	78.26

The close resemblance between the values measured experimentally and those predicted from the obtained model indicates the validity of the generated model. (Table 7).

***In-vitro* drug release of valsartan from SLN**

Release rate study

Phosphate buffer 6.8

Table. 8: Percent Cumulative Release of plain Valsartan

Time in (min)	Cumulative % drug release
10	10.4±0.86
20	19.3±0.59
30	29.7±1.20
40	34.1±0.95
50	40.5±1.85
60	46.9±0.72

Table. 9: Percent drug release of Valsartan from nanoparticles in PBS 6.8.

Time (in hours)	Cumulative % drug release (PBS 6.8)
0	0
1	10.28±0.17
2	17.71±0.85
3	25.66±0.26
6	35.64±1.03
9	50.85±0.17
12	63.72±0.40
18	72.94±0.26
24	79.55±0.32

Percent Cumulative Release curves: Drug release studies of Valsartan in its pure form and from nanoformulation were done in Phosphate buffer 6.8.

Plain Valsartan in Phosphate buffer 6.8

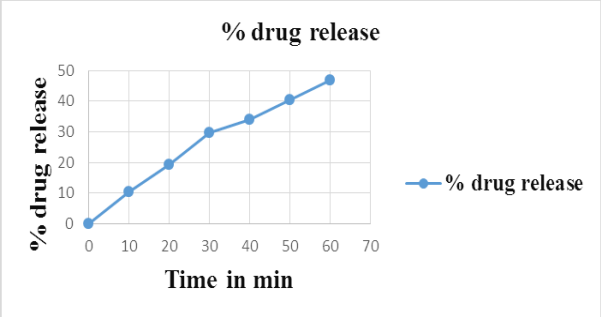


Fig. 10: Percent cumulative drug release profile of plain Valsartan.

Valsartan loaded SLN in Phosphate buffer pH 6.8: Formulation V-10.

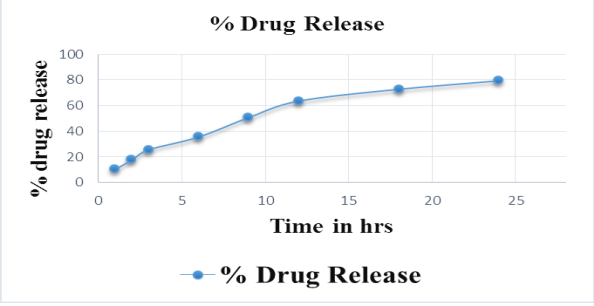


Fig. 11: Percent cumulative drug release profile of Valsartan from SLNs.

The release of V-10 SLN was found to be sustained when compared with the plain Valsartan. The formulation showed sustained release maximum upto 72-79% till 24 hours. When compared the release of plain Valsartan and that from the nanoformulation at 60 min the nanoformulation showed a sustained type of drug release. For application of kinetics to the release study, the release curve was divided in two parts as it showed biphasic behaviour. The initial burst release showed zero order kinetics (R^2= 0.76) while the second half of release curve was found to follow a Korsmeyer Peppas model (R^2= 0.98).

CONCLUSION

It was found that increase in concentration of lipids in combination with surfactant in formulation shows increase in entrapment efficiency and lower particles size. The initial burst release shown by the nanoformulation was due to the drug present on the surface of nanoparticles. When compared the release of plain Valsartan and that from the SLN (V-10) at 60 min the SLN showed a sustained type of drug release compared to plain Valsartan. From the above study it could be concluded that the Valsartan was an ideal candidate for formulating SLN as well as it was compatible with the excipients used. Thus V-SLN prove to be a good drug delivery system to overcome the previously mentioned problems.

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CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interests.

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