RESEARCH ARTICLE

Formulation and Evaluation of Controlled Release Maintenance Dose Loaded Niosomes of Anti-Hypertensive Drug

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ABSTRACT

The present study was aimed to formulate, comparatively evaluate and optimize multiple lipid drug carriers of valsartan for oral controlled release to overcome the problems associated with the drug such as bioavailability, to reduce the dosage regimen, half life and to determine the appropriateness of niosomal formulation as a drug carrier. Ether injection method was chosen for the formulation of physically and chemically stable niosomes of valsartan. The formulation and process parameters were optimized by manufacturing placebo niosomes. Than drug loaded niosome was prepared by varying the concentration of span 60. The prepared nine formulations were evaluated for various parameters. Placebo niosomes were evaluated for appearance, odour, texture, creaming volume, pH and changes after 15 days. The medicated nine formulations were evaluated for organoleptic properties (appearance/color, odour), pH, total drug content, entrapment efficiency, mean particle size and polydispersibility index, zeta potential and *In-vitro* drug release. All formulations were off-white in color, odourless, and fluid in nature. It was stable and did not show sedimentation. The pH was found to be in the range of 4.6-5.4. Drug content was found in the range of 89.13 to 99.52. The Entrapment efficiency was found in range of 79.05 to 98.24. The mean vesicle size of drug loaded niosomes of the different batches ranged between 2.52-3.42µm. The polydispersvity index was in the range of 0.325 to 0.420 which indicates a narrow vesicle size distribution. The values of zeta potential were in the range of -20.29 mV to -30.55 mV which indicates that niosome had sufficient charge and mobility to inhibit aggregation of vesicles. All the nine formulations shows constant drug release in controlled manner up to 24 h. Formulation V7 was considered to be the best formulation as the % drug content (99.52 ± 0.97), % entrapment efficiency (98.24 ± 1.50) and % drug release at the end of 24th h (98.55) were high for V7. The optimized formulation V7 showed higher degree of correlation coefficient (r^2) 0.9805 which indicates process of constant drug release from dosage form. The present study concludes that the prepared niosome is a convenient and efficiency carrier for the delivery of antihypertensive drug. Besides this, it provided controlled delivery of drug.

Keywords: Niosomes, Valsartan, Ether injection technique, Vesicular size, Controlled drug delivery.

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INTRODUCTION

Niosomes are non-ionic surfactant of the alkyl or dialkyl polyglycerol ether class and cholesterol with subsequent hydration in aqueous media. These are lamellar structures that are microscopic in size. Structurally niosomes are bilayered in nature. On the basis of preparation methods used, niosomes may be unilayer or multilayer. [1-2] Depending on various factors such as stability and cost, the niosomes are considered ideal when compared with liposomes. For various routes such as topical, ophthalmic and parenteral niosomes prove to be a potential and possible drug delivery mechanism. [3] It was prepared by the method of ether injection method, handshaking method (thin film hydration technique), sonication, microfluidization, multiple membrane extrusion method, reverse phase evaporation technique, transmembrane pH gradient (inside acidic) drug uptake process (remote loading) and the "Bubble" method. [4-8] Various factors affecting niosomal formulations are type of surfactant, drug and amount, charge and cholesterol content, osmotic stress due to resistance and constitution of membrane. [9-12]

The main aim of this project is to study about niosome which is emerging as a potential drug carrier, as a new drug delivery for the antihypertensive drug Valsartan. Valsartan a widely prescribed anti hypertensive drug belongs to class II under BCS classification and exhibit low and variable bioavailability due to its aqueous solubility and it has a shorter half life (6 h). [13] The oral bioavailability of Valsartan was reported to 23%. [14] Due to this it require frequent dosing to maintain the therapeutic blood level of the drug for long term treatment. Therefore, the objective behind the study is to sustain the action by incorporating the maintenance dose of drug into the vesicular carriers. [15]

MATERIALS AND METHODS

Chemicals and reagents

Valsartan was obtained as gift sample from Torrent research centre, Ahmedabad, India. Sorbitan monostearate (Span 60) and Diethyl ether were purchased from Merck limited, Mumbai, India. Cholesterol was purchased from Loba chemie, Boisar, India. All the other reagents and chemicals were of analytical grade.

Instruments

Electronic Weighing Balance (Mettlet Toledo, XP205, B047091022), Magnetic stirrer (Remi, 2 MLH), Sonicator (Life care instruments pvt. ltd.), UV Spectrophotometer (Lab India, UV3092, 20-1950-21-0004), FTIR Spectrophotometer (Bruker, Alpha, 200430), Differential Scanning Colorimeter (DSC) (Shimadzu, DSC 60), Hot air oven (Equichem, 1109/07) and pH meter (Eutech, pH2700, 739092) were used in present work.

Preformulation study of drug

Pre-formulation investigations are to provide information on physicochemical and biopharmaceutical study properties of drug molecule, non-drug substance and materials used for packaging as well as compressibility

Characterization of Pure drug

Valsartan was obtained as a gift sample from Torrent research centre, Ahmedabad and were subjected to following characterization tests.

Determination of λ_{max} by UV spectroscopy

Using Methanol, the absorption maxima of Valsartan was obtained. A range of solutions (2-10µg/ml) was scanned using UV spectrophotometer.

Melting point determination

Melting point of Valsartan was determined using capillary tube method. Observed value was compared with the reported value.

FTIR spectroscopy

The IR spectrum of Valsartan was recorded using Fourier transform infrared spectrophotometer with diffuse reflectance principle by KBr press pellet method.

Loss on Drying

A sample of 0.1 g of Valsartan was weighed individually in a vessel. The sample containing vessel was then placed in an hot air oven at 100°C for 4 h. The sample was cooled in a desiccator and weighed. [16]

DSC study

The DSC thermogram of Valsartan was recorded using Differential scanning calorimeter. Approximately 2 to 5 mg of sample will be heated in a closed pierced aluminum pan from 30°C to 180°C at a heating rate of 5°C/min under a stream of nitrogen at a flow rate of 50 ml/min.

Equilibrium solubility study

The shake flask method was used to determine saturation solubility of Valsartan in different solvent (i.e. Methanol, 0.1N HCl pH 1.2, Phosphate buffer pH 6.8 and Phosphate buffer pH 7.2).

% Purity

For % purity, 100 mg of Valsartan were individually weighed and transferred to a 100 ml of volumetric flask. Then the volume was made up to 100 ml mark with methanol. The flask was kept on a sonicator individually for 5 min. Solution was then filtered using a whatman filter paper. Then aliquot 10 ml from the filtered solution and dilute it up to 100 ml using methanol. Then absorbance of the resulting solution was measured at the λmax 250 nm for Valsartan using UV-Visible double beam spectrophotometer against methanol as blank. The linearity equation obtained from calibration curve was used for estimation of purity of Valsartan. [16]

Powder characterization (physical properties)

Valsartan was characterized by Angle of repose, Bulk density, Tapped density, Carr's index and Hausner's ratio. [16-18]

Drug excipients compatibility studies

The drug Valsartan and excipients must be compatible with one another to produce a product i.e. stable, efficacious, attractive, easy to administer and safe. The

compatibility study of the drugs: excipients in physical mixture (1:1) were checked out using the shimadzu FTIR spectrophotometer by KBr press pellet method. [19] The FTIR spectra of pure drugs and with the excipients are shown in Fig. 3 and Fig. 4.

Analytical method development and validation

Analytical measurement of Valsartan in Methanol by UV spectrophotometry was validated separately as per ICH guideline, Q2(R1). The UV spectrophotometric method was validated for the quantification of Valsartan in samples. Intraday and Interday precision and accuracy were determined by analysis of five concentrations. The overall precision of the method was expressed as relative standard deviation (RSD) and the accuracy of the method was expressed in terms of relative error. [20]

Preparation of standard curve of Valsartan in Methanol at 250 nm

Preparation of standard stock solution (1000µg/ml)

Accurately weighed 100 mg of Valsartan was dissolved in 100 ml of Methanol to get a concentration 1000µg/ml. This course of action was then used for arranging working standard plan.

Preparation of working standard solution (100µg/ml)

10 ml standard stock solution of Valsartan was transferred to a 100 ml volumetric flask and volume was adjusted to 100 ml with Methanol to get a concentration of 100µg/ml.

Preparation of dilutions for calibration curve

With appropriate dilution of working standard solution 5, 10, 15, 20, 25, 30, 35 and 40µg/ml concentration of Valsartan was obtained. The absorbance of prepared solutions of Valsartan in Methanol was measured at wavelength maximum 250 nm using Shimadzu UV-1800 spectrophotometer against Methanol as blank. The experiment was performed in triplicate and based on average absorbance; the equation for the best line was generated. The results of standard curve preparation are shown in Table 6 and Fig. 5.

Validation of analytical method of Valsartan in Methanol

Analytical measurement of Valsartan in Methanol by UV spectrophotometry was validated as per ICH guideline, Q2AR1.

Linearity and range

Linearity is expressed in terms of correlation coefficient of linear regression analysis. The linearity response was determined by analyzing 8 independent levels of calibration curve in the range of 5-40µg/ml. Plot the calibration curve of absorbance vs concentration and determines correlation coefficient and regression line equations for Valsartan.

Accuracy preparation of sample solution

The accuracy study was determined by standard addition method. 100 mg of Valsartan was weighed and transferred into a 100 ml of volumetric flask, dissolved and diluted up to mark with Methanol. Pipette out 10 ml of the above solution in 100 ml volumetric flask and diluted to mark with Methanol to

get 100µg/ml solution of Valsartan, from that 10µg/ml of solution was prepared. To one ml of the above solution, increasing aliquots of standard solution (5, 10 and 15µg/ml of Valsartan) was added and diluted to 10 ml with Methanol. Absorbance of solution was measured at selected wavelength. The amount of Valsartan was calculated at each level and % recoveries were computed.

Precision

Repeatability: The absorbance of same concentration was measured three times and RSD was calculated.

Intraday Precision: Solutions containing 5-40µg/ml of Valsartan was analyzed three times on the same day and % RSD was calculated.

Interday Precision: Solutions containing 5-40µg/ml of Valsartan was analyzed three times on the different 3 d and % RSD was calculated. It is a measure of either the degree of reproducibility or repeatability of the analytical method.

LOD

The limit of detection (LOD) is the lowest amount of analyte in a sample that can be detected, but not necessarily quantified, under standard experimental condition. LOD will be calculated using the following formulae:

$$LOD = 3.3\ \sigma/S$$

Where σ is Standard deviation of the response and S is slope of the calibration curve.

LOQ

The limit of quantification (LOQ) is the lowest amount of analyte in a sample that can be determined with acceptable precision and accuracy under standard experimental condition. LOQ were calculated using the following formulae:

$$LOQ = 10\sigma/S$$

Where σ is Standard deviation of the response and S is slope of the calibration curve.

Formulation development of Niosome

Quality Target Product Profile for the finished dosage form

Based on properties of drug substance, intended patient population and literature studies Quality target product profile (QTPP) was defined for finished dosage form Niosome. [21]

Critical quality attributes (CQAs) of finished dosage form based on QTPP

Based on severity of harm to a patient (safety and efficacy) resulting from failure to meet that quality attribute of the finished dosage form critical quality attributes (CQAs) was defined. [22]

Dose calculation for loading and maintenance dose of Valsartan

Oral dose (X_o): 80 mg

Dosing interval (τ): 24 h

Elimination half life $(t_{1/2})$: 6 h

Elimination rate constant $(Ke) = \dfrac{0.693}{t_{1/2}} = \dfrac{0.693}{6} = 0.1155$

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Table 1: Quality Target Product profile (QTPP) for Niosomes

QTPP Element	Target	Justification
Dosage form	Niosomes	Higher stability, Low cost and Sustained effect
Dosage design	Sustained release	Hypertension is a widespread condition of the high systemic arterial pressure and it is an important factor for the extension of cardiovascular diseases. Valsartan loaded niosomes for sustained release that will help to maintain therapeutic concentration of drug and also reduces the frequency of dosing and give better patient compliance.
Route of administration	Oral	Better patient compliance
Dosage strength	51.14 mg Valsartan (maintenance dose)	To maintain therapeutic concentration of drug for prolonged period of time
Stability	At least 24 mo shelf life at room temperature	Maintain Quality, safety and efficacy throughout product life cycle.
Drug product quality attributes	Organoleptic properties, pH, total drug content, Entrapment efficiency, Mean particle size, Polydispersibility index, Zeta potential and *In-Vitro* drug release	Meeting the same compendia or other applicable (quality) standard
Container closure system	Suitable container closure system	Needed to achieve the target shelf-life and to ensure finished dosage form integrity during shipping

QTPP: Quality Target Product profile

Table 2: Critical quality attributes (CQAs) of Niosomes

Quality attributes of the finished dosage form	Target	Is it CQA?	Justification
Organoleptic properties (Colour, Odour)	Should be acceptable to patient	No	Color, Odour and appearance are not directly linked to safety and efficacy. Therefore, they are not critical. The target is set to ensure patient acceptability.
pH	Should meet the relevant pharmacopoeia	No	pH is not directly linked to safety and efficacy. Therefore, they are not critical.
Total drug content	100.0 % of label claim	Yes	Drug content variability should affect safety and efficacy. Process variables may affect the drug content of the finished dosage form. Hence, it is critical parameter.
Entrapment efficiency	Should have maximum drug entrapment	Yes	Entrapment efficiency should have impact on drug release. Hence, it is critical parameter.
Mean particle size, Polydispersibility index	Should have narrow vesicle size distribution	Yes	Particle size affect the uniform distribution of API and drug release. Hence, it is critical parameter.
Zeta potential	Should have sufficient charge	Yes	Zeta potential affect the mobility to inhibit aggregation of niosomes. Hence, it is critical parameter.
In-Vitro drug release	Should have maximum drug release at 24th h	Yes	Failure to meet the *In-Vitro* drug release specification can impact bioavailability. Both formulation and process variables affect the dissolution profile. Hence, it is critical parameter.

CQA: Critical quality attributes

$$D_L = \frac{C_{ss} \cdot V_d}{F}$$

But, $C_{ss} = \frac{F \cdot X_o}{K_e \cdot V_d \cdot \tau}$

Thus, $D_L = \frac{F \cdot X_o \cdot V_d}{K_e \cdot V_d \cdot \tau \cdot F}$

$$D_L = \frac{X_o}{K_e \cdot \tau}$$

$$D_L = \frac{80}{0.1155 \cdot 24}$$

$D_L = 28.86$ mg

$$D_T = D_L \left(1 + \frac{0.693 * \tau}{t_{1/2}}\right)$$

$$D_T = 28.86 \left(1 + \frac{0.693 * 24}{6}\right)$$

$$D_T = 28.86 \left(1 + \frac{16.632}{6}\right)$$

$D_T = 28.86 \times 3.772$

$D_T = 79.99$ mg = 80.00 mg

$D_T = D_L + D_M$

$80.00 = 28.86 + D_M$

$D_M = D_T - D_L$

$D_M = 80.00 - 28.86 = 51.14$ mg

Where,

D_L = Loading dose

D_M = Maintenance dose

D_T = Total dose

C_{ss} = Steady state concentration

τ = Time for intended release

$t_{1/2}$ = Biological half life

V_d = Volume of distribution

Preparation of Niosomes by Ether injection method
Preparation of Placebo Niosomes
Description of manufacturing process

The various concentration ranges of non ionic surfactant Span 60 and Cholesterol were weighted accurately and dissolved in 10 ml of ether in a small beaker. In another beaker 10 ml phosphate buffer pH 6.8 was taken. Then the dissolved surfactant/lipid solution was taken into a syringe and injected slowly at a different rate of addition, via a 24 gauge needle in phosphate buffer pH 6.8 which was magnetically

stirred at different RPM continuously and maintained at different temperature for different time. As the lipid solution was injected slowly into the aqueous phase, vaporization of ether leads to the formation of niosomes.

Preliminary characterization of placebo niosomes
Organoleptic properties
The placebo niosomes were evaluated for colour, odour, appearance and texture.

Creaming volume
The placebo niosomes were kept undisturbed for 24 h in measuring cylinder and evaluated for separation, creaming and redispersibility.

pH
The pH of placebo niosomes was checked with digital pH meter.

Changes after 15 d
The placebo niosomes were kept undisturbed for two weeks and were observed for any changes in the formulations.

Effect of Critical formulation parameters and processing parameters on formation of niosomes
After optimizing the type of span and cholesterol in the preparation of Niosomes; the processing parameters such as rate of addition, stirring speed, stirring time and stirring temperature were optimized.

Type of non-ionic surfactant
Different type (grade) of Span was used for formulation of niosomes. Optimize the concentration in niosome using maximum drug entrapment.

Surfactant: Cholesterol ratio
The surfactant cholesterol ratio is very important to optimize because the cholesterol acts as stabilizer and itself is lipophilic in nature so increased concentration of cholesterol may cause reduction in drug entrapment. The optimum concentration is needed otherwise vesicle stability may decrease. Optimize the ratio by niosomes having maximum drug entrapment.

Effect of Rate of addition
The ether solution was injected rapidly as well as dropwise into aqueous phase to study its effect on vesicle size. Optimize the rate of addition by distribution size of niosomes.

Effect of Stirring speed and time
The dispersion was stirred for varied speed and time. Optimize the rate of addition by distribution size of niosomes.

Effect of Stirring temperature
The dispersion was stirred at different temperature. Optimize the rate of addition by distribution size of niosomes.

Optimization & Preparation of drug loaded niosomes (Medicated Niosomes)
After optimization of formulation and process parameters medicated niosomes was prepared. The various concentration ranges of non ionic surfactant Span 60 and Cholesterol were weighted accurately and dissolved in 10 ml of ether in a small beaker. In another beaker 10 ml phosphate buffer pH 6.8 and 51.14 mg

active pharmaceutical ingredient Valsartan were taken. Then the dissolved surfactant/lipid solution was taken into a syringe and injected slowly at a rate of 0.25 ml/min, via a 24 gauge needle in phosphate buffer pH 6.8 containing Valsartan which was magnetically stirred at 500 rpm continuously and maintained at 60°C-65°C for 30 min. As the lipid solution was injected slowly into the aqueous phase, vaporization of ether leads to the formation of niosomes.

Table 3: Formulations of Valsartan loaded Niosomes

S. No.	Formulation code	Valsartan (%)	Span 60 (%)	Cholesterol (%)
1.	V1	1.0	0.5	1.0
2.	V2	1.0	1.0	1.0
3.	V3	1.0	1.5	1.0
4.	V4	1.0	2.0	1.0
5.	V5	1.0	2.5	1.0
6.	V6	1.0	3.0	1.0
7.	V7	1.0	3.5	1.0
8.	V8	1.0	4.0	1.0
9.	V9	1.0	4.5	1.0

Note: All formulation contains 10 ml diethyl ether.

Evaluation parameters for Medicated Niosomes
Organoleptic properties
The medicated niosomes were evaluated for colour, odour and appearance.

pH
The pH of medicated niosomes was checked by digital pH meter.

Total drug content
Assay of medicated niosomes were carried out by U.V. method. Two ml of niosome was dissolved into 50 ml Phosphate buffer solution having pH 6.8. The sample was stirred at 100 rpm to break the niosomes. Drug content was determined using UV spectrophotometer at respective absorption maxima.

Mean particle size and Polydispersibility index
The particle size analysis of the formulation (V7) was determined using Beckman particle size determination technique. The graph of particle size distribution was shown in Fig. 10.

Entrapment efficiency
Unentrapped drug from niosome was separated by centrifugation method. Niosomes were centrifuged at 20,000 rpm at controlled temperature of 4°C for 60 min. By using UV spectroscopy unentrapped drug was quantified at respective absorption maxima. The results of medicated niosomes were shown in Table 17.

Zeta potential
Zeta potential of the dispersion was determined by Malvern zetameter. Time duration for zeta potential determination was 60 s and charge was find out. Typical graphs for zeta potential of medicated niosomes were shown in Fig. 11.

In-vitro Drug Release Studies
The release of valsartan from niosomes was determined by using membrane diffusion technique. The niosomal formulation equivalent to 51.14 mg of valsartan was placed in a glass tube of diameter 2.5 cm with an

effective length of 8 cm which was tied with previously soaked cellulose membrane (12,000–14,000 Da Molecular weight cut off), which acts as a donor compartment. The glass tube was placed in a beaker containing 100 ml of phosphate buffer (pH 6.8), acting as a receptor compartment. The whole assembly was fixed in such a way that the lower end of tube containing suspension was just touching (1-2 mm depth) the surface of diffusion medium. The temperature of receptor medium was maintained at 37 ± 5°C and was agitated at the speed of 100 rpm using magnetic stirrer. Aliquots of 5 ml sample were withdrawn periodically and after each withdrawal same volume of medium was replaced. The collected samples were analyzed at 250 nm in double beam UV-VIS spectrophotometer using Phosphate Buffer (pH 6.8) as blank. [23]

Optimization Procedure: Among the nine formulations prepared, optimization of the best formulation was done based on the *In-vitro* drug release, total drug content and % entrapment efficiency results.

Table 4: Results of characterization of pure drugs

S. No	Test Parameters	Results
1.	Physical Appearance	A white powder
2.	λmax by UV spectroscopy (In Methanol)	250 nm
3.	Melting point	102°C -107°C
4.	Loss on Drying	0.41% (NMT 0.5%)
5.	Equilibrium solubility study (mg/ml)	Methanol 0.653
		Water 0.160
		0.1 N HCl, pH 1.2 0.081
		Phosphate buffer, pH 6.8 1.321
		Phosphate buffer, pH 7.4 1.384
6.	% Purity	99.86% (98.0% - 102.0%)
7.	Powder characterization	
	Angle of Repose (°)	16.46
	Bulk density (g/ml)	0.4137
	Tapped density (g/ml)	0.5578
	Carr's index	42.361
	Hausner's ratio	1.7328

RESULTS AND DISCUSSION
Preformulation study
Characterization of Pure drugs

From the above results it was concluded that all the practical results were complied with theoretical values. The melting point of Valsartan was found to be 102°C-107°C which complies with theoretical values thus indicating purity of obtained drug sample. Valsartan has poor solubility in 0.1N HCl but its permeability was found to be more towards acidic side. Hence further emphasis was given to evaluate the drug dissolution at this particular media along with pH 6.8 buffer as its solubility was found to be comparatively more at this particular media. From the solubility study data Valsartan also shows lower solubility in water. Powder characteristics indicate that Valsartan has good flowability.

FTIR spectroscopy

The FTIR spectrum (Fig. 1) of Valsartan individually revealed characteristic peaks which are shown in Table 5.
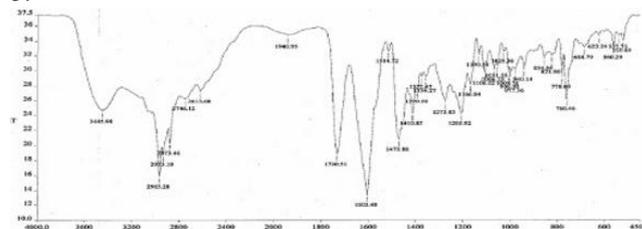
Fig. 1: FTIR Spectrum of Valsartan pure drug

Table 5: FTIR characteristic peaks of Valsartan pure drug

Reference peaks (cm^{-1})	Observed peaks (cm^{-1})	Inference
1593.00	1598.99	N=N bending (Aromatic secondary amine)
1410.00	1409.96	C=C stretching
2870.00	2872.01	C-H stretching (Alkane)
1730.00	1726.29	C=O stretching (Acyclic saturated)

FTIR spectra of the Valsartan were recorded in the range of 400-4000 cm^{-1}. The principal IR peaks were all observed at 1598.99 cm^{-1}, 1409.96 cm^{-1}, 2872.1 cm^{-1} and 1726.29 cm^{-1} in the above spectra of Valsartan. These observed principal peaks were comparable to the reference peaks of the Valsartan. This observation confirmed the purity and authenticity of the Valsartan.

Differential scanning Calorimetry (DSC) study

In order to confirm physical state of Valsartan in finished dosage form, DSC of Valsartan was carried out and showed in Fig. 2.
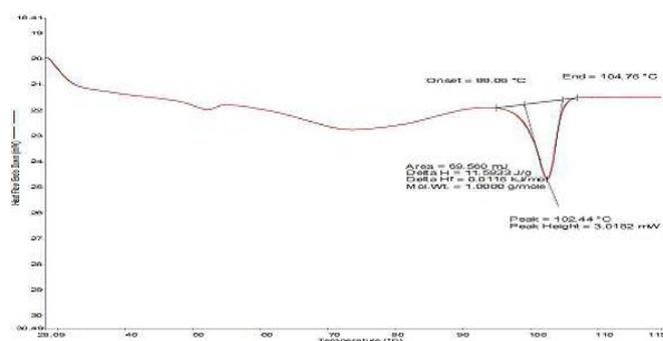
Fig. 2: DSC graph of Valsartan pure drug

DSC thermogram of Valsartan was recorded in the range of 20-119°C. The DSC thermogram of Valsartan showed sharp endothermic peak at its melting point of 104.76°C. This observation confirmed the purity and authenticity of the Valsartan.

Drug excipients compatibility studies
Physical change

The samples were checked for physical changes such as liquefaction, discoloration, odour and no changes observed during compatibility study.

Fourier Transform Infrared Spectroscopy (FTIR)

There was no shift in the characteristic peak of drug in the spectra of drug: drug and drug: excipients. FTIR

Spectrophotometric analysis shows no evidence of interaction between drug and studied excipients.
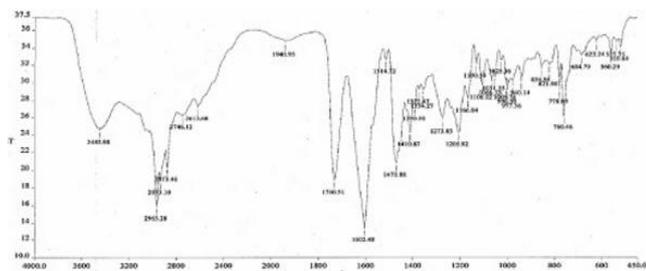
Fig. 3: FTIR Spectrum of Valsartan pure drug

Fig. 4: FTIR Spectrum of Valsartan + Cholesterol

Analytical method development and validation
Preparation of standard curve of Valsartan

The drug is freely soluble in methanol hence it is chosen as a solvent for developing the method. Valsartan exhibits maximum absorbance at 250 nm and obeyed Beer's law in the range of 5-40µg/ml. The results of calibration curve preparation were showed in Table 6 and Fig. 5.

Table 6: Standard calibration curve data of Valsartan in Methanol at λmax 250 nm

S. No.	Concentration (ug/ml)	Absorbance			Absorbance (Mean* ± SD)
1	5	0.103	0.102	0.104	0.103 ± 0.001
2	10	0.215	0.219	0.219	0.217 ± 0.0023
3	15	0.319	0.318	0.321	0.319 ± 0.0015
4	20	0.454	0.456	0.453	0.454 ± 0.0015
5	25	0.592	0.59	0.592	0.592 ± 0.0012
6	30	0.763	0.762	0.76	0.761 ± 0.0015
7	35	0.92	0.92	0.921	0.920 ± 0.0005
8	40	1.234	1.233	1.235	1.234 ± 0.001

SD: Standard deviation, *: Mean of each 3 reading
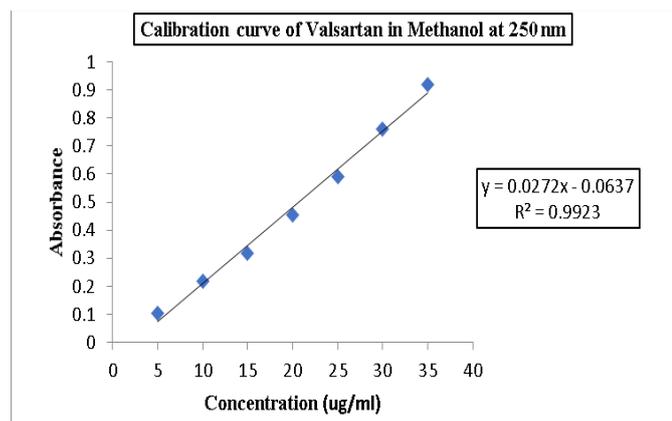
Fig. 5: Standard calibration curve of Valsartan in Methanol

Validation of analytical method of Valsartan
Linearity and range

Valsartan exhibits maximum absorbance at 250 nm and obeyed Beer's law in the range of 5-40µg/ml. The results of calibration curve preparation were showed in Table 6 and Fig. 5.

Accuracy (% recovery)

The % recoveries obtained were 98.47%–108.07%. The results of recovery study were showed in Table 7.

Table 7: Data of recovery study for Valsartan in Methanol at λmax 250 nm

S. No	Amount of drug taken (µg/ml)	Amount of drug added (µg/ml)	Total amount of drug (µg/ml)	Amount of drug found (µg/ml)	% Recovery
1	10	-	10	-	-
2	10	5	15	14.77	98.47
3	10	10	20	20.68	103.44
4	10	15	25	27.01	108.07

Precision

The % RSD found 0.123%-1.473% for intraday and 0.046%-1.459% for interday.

Precision revealed that the proposed method is precise. Results were showed in Table 8 and Table 9.

Table 8: Intraday precision data for Valsartan in Methanol at 250 nm

S. No	Concentration (µg/ml)	Absorbance			Absorbance (Mean*± SD)	% RSD
		1	2	3		
1	5	0.102	0.104	0.105	0.104 ± 0.0015	1.473
2	10	0.22	0.218	0.217	0.218 ± 0.0015	0.699
3	15	0.321	0.324	0.325	0.323 ± 0.0020	0.643
4	20	0.456	0.454	0.453	0.454 ± 0.0015	0.336
5	25	0.591	0.589	0.588	0.589 ± 0.0015	0.259
6	30	0.759	0.763	0.762	0.761 ± 0.0021	0.273
7	35	0.919	0.921	0.923	0.921 ± 0.002	0.217
8	40	1.236	1.233	1.235	1.234 ± 0.0015	0.123

SD: Standard deviation, RSD: Relative standard deviation, *: Mean of each 3 reading

Table 9: Interday precision data for Valsartan in Methanol

S. No	Concentration (µg/ml)	Absorbance			Absorbance (Mean*± SD)	% RSD
		1	2	3		
1	5	0.103	0.106	0.105	0.105 ± 0.0015	1.459
2	10	0.216	0.215	0.213	0.215 ± 0.0015	0.711
3	15	0.318	0.32	0.319	0.319 ± 0.001	0.313
4	20	0.452	0.45	0.453	0.452 ± 0.0015	0.338
5	25	0.589	0.588	0.591	0.589 ± 0.0015	0.259
6	30	0.761	0.759	0.76	0.760 ± 0.001	0.131
7	35	0.923	0.921	0.924	0.923 ± 0.0015	0.165
8	40	1.231	1.23	1.231	1.231 ± 0.0005	0.046

SD: Standard deviation, RSD: Relative standard deviation, * : Mean of each 3 reading

Limit of detection (LOD)

Limit of detection (LOD) of Valsartan was found 7.522µg/ml.

Limit of quantitation (LOQ)

Limit of quantitation (LOQ) of Valsartan was found 22.795µg/ml.

Summary of Validation Parameters

The results of the analysis of Valsartan by the proposed method were highly reproducible, and reliable which conclude that the proposed method is highly simple, sensitive, reproducible, economic, less time consuming and easy to apply for routine analysis of Valsartan in Methanol.

Table 10: Summary of validation parameters of Valsartan in Methanol

S. No	Validation parameters	Results
1	Linearity range (µg/ml)	5-40
2	Linearity equation	y=0.0272x-0.0637
3	Linearity (R2, Correlation coefficient)	0.9923
4	Precision (% Intraday RSD)	0.123%-1.473%
	Interday	0.046%-1.459%
5	Accuracy (% Recovery)	98.47% – 108.07%
6	LOD (µg/ml)	7.522
7	LOQ (µg/ml)	22.795

RSD: Relative standard deviation, LOD: Limit of detection, LOQ: Limit of quantitation

Table 11: Observation of placebo niosomes

S. No.	Batch No.	Span 60 (%)	Cholesterol (%)	Result
1.	T1	0.1	0.5	Dispersion was not obtained
2.	T2	0.2	0.5	Dispersion was not obtained
3.	T3	0.3	0.5	Dispersion was not obtained
4.	T4	0.4	0.5	Dispersion was not obtained
5.	T5	0.5	0.5	**Milky white dispersion was obtained**
6.	T6	0.6	0.5	Separation was obtained
7.	T7	0.7	0.5	Separation was obtained
8.	T8	0.8	0.5	Separation was obtained
9.	T9	0.9	0.5	Separation was obtained
10.	T10	1.0	0.5	Separation was obtained
11.	T11	0.1	1	Dispersion was not obtained
12.	T12	0.2	1	Dispersion was not obtained
13.	T13	0.3	1	Dispersion was not obtained
14.	T14	0.4	1	Dispersion was not obtained
15.	T15	0.5	1	**Milky white dispersion was obtained**
16.	T16	1.0	1	**Milky white dispersion was obtained**
17.	T17	2.0	1	**Milky white dispersion was obtained**
18.	T18	3.0	1	**Milky white dispersion was obtained**
19.	T19	4.0	1	**Milky white dispersion was obtained**
20.	T20	5.0	1	Separation was obtained
21.	T21	6.0	1	Separation was obtained
22.	T22	7.0	1	Separation was obtained
23.	T23	0.1	1.5	Dispersion was not obtained
24.	T24	0.2	1.5	Dispersion was not obtained
25.	T25	0.3	1.5	Dispersion was not obtained
26.	T26	0.4	1.5	Dispersion was not obtained.
27.	T27	0.5	1.5	**Milky white dispersion was obtained**
28.	T28	1.0	1.5	**Milky white dispersion was obtained**
29.	T29	2.0	1.5	Separation was obtained
30.	T30	3.0	1.5	Separation was obtained

Formulation development
Placebo Niosomes

Non-ionic surfactant concentration from 0.1-0.4% w/v did not give proper dispersion this may be due to insufficient concentration of non-ionic surfactant to form uniform spherical vesicle. Whereas surfactant concentration more than 0.6% w/v with 0.5% w/v cholesterol, 5.0% w/v with 1.0% w/v cholesterol and 2.0% w/v with 1.5% w/v cholesterol showed cracking this may be due to precipitation of surfactant.

Batch no. T5, T15-T19, T27-T28 gave milky white dispersion in which the concentration of surfactant was in the range of 0.5-4.5% w/v and hence, this were taken for the further optimization with 1% Cholesterol concentration.

Table 12: Physical properties of placebo niosomes

Batch No	Appearance/ Colour	Odour	Texture	Creaming volume	pH	Changes after 15 d
T-5	Milky white	Odourless	Smooth	No change	4.5-5.6	No changes
T15-T19	Milky white	Odourless	Smooth	No change	4.5-5.6	No changes
T27-T28	Milky white	Odourless	Smooth	No change	4.5-5.6	No changes

Table 13: Effect of processing parameters on particle size distribution

Parameters	Effect on particle size distribution
Rate of addition	
Rapid addition	Non uniform size distribution
Drop wise addition during min	Uniform size distribution
Stirring speed	
250 RPM	Non uniform size distribution
300 RPM	Non uniform size distribution
500 RPM	Uniform size distribution
Stirring time	
5 min	Non uniform size distribution
10 min	Non uniform size distribution
20 min	Non uniform size distribution
30 min	Uniform size distribution
Stirring temperature	
50°C-55°C	Non uniform size distribution
60°C-65°C	Uniform size distribution
65°C-70°C	Non uniform size distribution

Table 14: Final selected chemical and processing parameters for formulation of Niosomes

Optimized parameters	
Concentration of Span 60	0.5% w/v-4.5% w/v
Concentration of Cholesterol	0.1% w/v
Rate of addition	Drop wise addition during min
Stirring speed	500 RPM
Stirring time	30 min
Stirring temperature	60°C-65°C

Fig. 6: Preparation of medicated niosome

Fig. 7: Prepared placebo and Optimized V7 formulations

Therefore by carried out placebo niosome batches we have optimized chemical parameters and processing parameters. Chemical parameters i.e. concentration of span 60 & cholesterol and processing parameters include rate of addition, stirring speed, stirring time and stirring temperature.

Evaluation parameters for Medicated Niosomes
Organoleptic properties
The Valsartan niosomes were off-white in color, odourless, and fluid in nature. It was stable and did not show sedimentation. The results of Organoleptic properties i.e. Appearance, Colour and Odour for all the nine batches are summarize in below Table 15.

Table 15: Organoleptic properties of medicated niosome

Batch No	Appearance/Colour	Odour
V1	Milky white	Odourless
V2	Milky white	Odourless
V3	Milky white	Odourless
V4	Milky white	Odourless
V5	Milky white	Odourless
V6	Milky white	Odourless
V7	Milky white	Odourless
V8	Milky white	Odourless
V9	Milky white	Odourless

Table 16: pH and Total drug content of medicated niosome

Batch No	pH	Drug content (% ± SD)
V1	4.7	89.23 ± 1.75
V2	4.6	90.20 ± 0.61
V3	5.1	89.13 ± 0.79
V4	4.9	95.41 ± 0.90
V5	4.7	97.76 ± 1.50
V6	5.2	97.29 ± 0.59
V7	5.4	**99.52 ± 0.97**
V8	5.1	97.93 ± 1.25
V9	4.8	98.45 ± 1.19

SD: Standard deviation

pH and Drug content
pH was found to be in the range of 4.6-5.4. The drug content was found in the range of 89.13 to 99.52. The results of pH value and Drug content for all the nine batches are summarize in below Table 16.

Entrapment efficiency, Mean particle size and Polydispersibility index, Zeta potential
The Entrapment efficiency was found in range of 79.05 to 98.24. Formulation V7 shows highest % entrapment efficiency values. The mean vesicle size of drug loaded

niosomes of the different batches ranged between 2.52-3.42µm. The polydispersvity index (PdI) was in the range of 0.325–0.420 for drug loaded niosomes which indicates a narrow vesicle size distribution. The values of Zeta potential of the drug loaded niosomal formulation were in the range of -20.29 to -30.55 mV. Values of zeta potential showed that the medicated niosomes had sufficient charge and mobility to inhibit aggregation of vesicles.
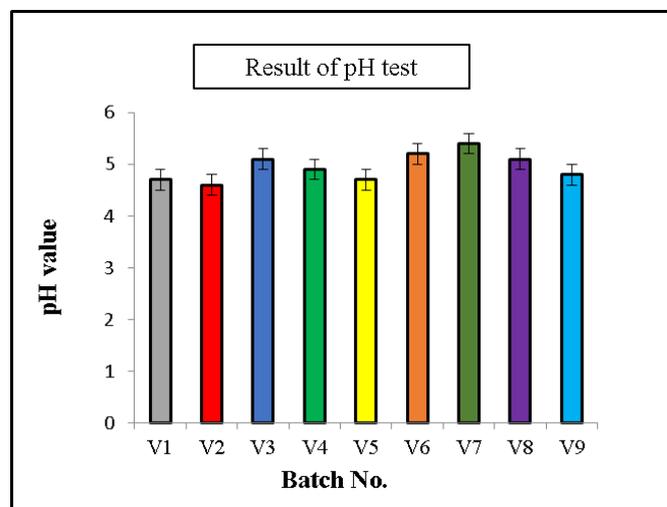
Fig. 8: Bar diagram of pH values of various niosome batches
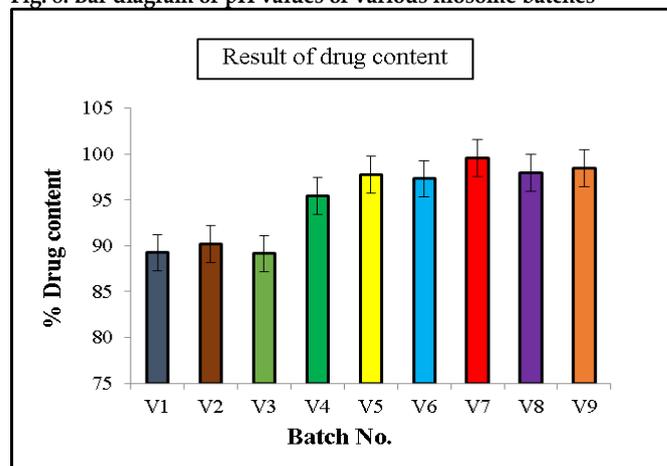
Fig. 9: Bar diagram of Total drug content of various niosome batches

The results of entrapment efficiency, mean particle size and polydispersibility index and zeta potential for all the nine batches are summarize in below Table 17.

Table 17: Entrapment efficiency of medicated niosome

Batch No.	Entrapment Efficiency (%) ± SD	Polydispersibility index	Particle size (µm) ± SD	Zeta potential (Mv)± SD
V1	85.52 ± 1.13	0.411	2.76 ± 0.84	-27.77 ± 1.55
V2	83.60 ± 1.39	0.389	2.99 ± 0.97	-24.84 ± 0.79
V3	79.05 ± 1.14	0.420	3.24 ± 0.86	-20.29 ± 1.03
V4	89.60 ± 2.26	0.385	3.08 ± 0.55	-25.44 ± 0.92
V5	88.09 ± 1.94	0.325	3.20 ± 0.90	-21.07 ± 1.75
V6	84.84 ± 1.60	0.370	3.42 ± 0.77	-24.57 ± 0.16
V7	**98.24 ± 1.50**	**0.387**	**2.52 ± 1.50**	**-25.69 ± 1.87**
V8	93.24 ± 2.25	0.404	2.90 ± 0.60	-28.27 ± 0.28
V9	90.16 ± 1.03	0.395	3.32 ± 0.61	-30.55 ± 0.28

SD: Standard deviation
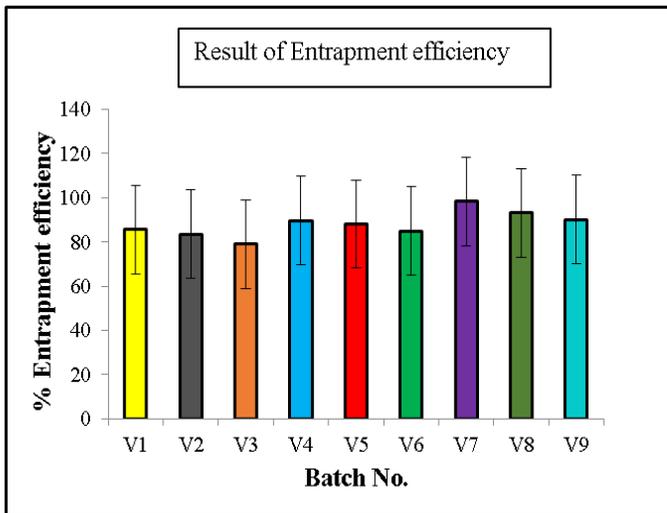
Fig. 10: Bar diagram of Entrapment efficiency values of various niosome batches
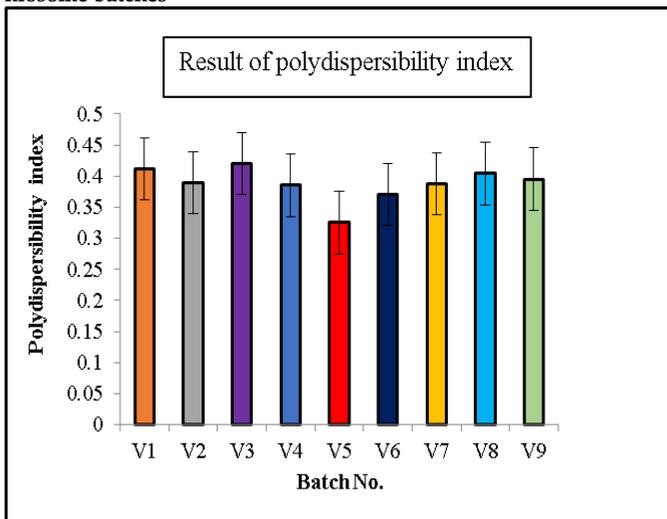
Fig. 11: Bar diagram of Polydispersibility index values of various niosome batches
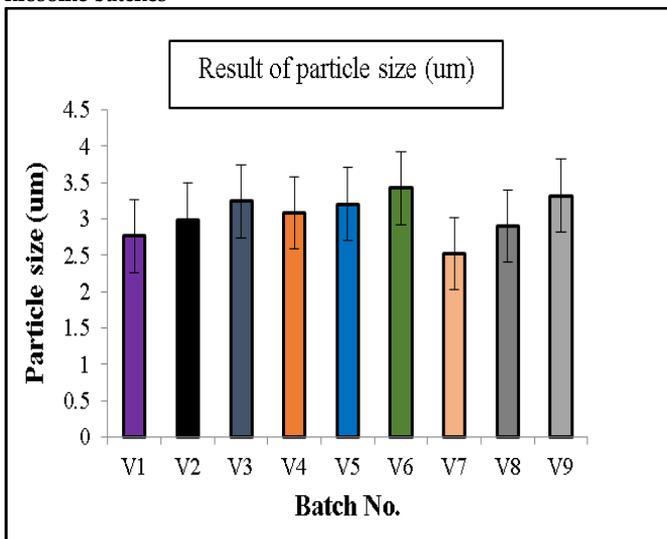
Fig. 12: Bar diagram of Particle size values of various niosome batches

In-vitro **drug release studies**

For all the nine formulations there was no initial burst release but the release was constant in a controlled manner for a period of time upto 24 h. The best formulation is the one which provides good

morphology (size and shape), high drug content, entrapment efficiency and controlled and prolonged drug release. Formulation V7 was considered to be the best formulation as the drug content, entrapment efficiency and the percent drug release were high for V7. This is the niosomal formulation containing comparatively high amount of surfactant prepared by ether injection method. The results of *in-vitro* drug release revealed that the drug was released in a controlled manner from all the formulations and V7 showed maximum drug release at the end of 24th h. Hence from all the above results of morphology, drug content, entrapment efficiency and *in-vitro* drug release studies, it is proved that formulation V7 is the best and optimized formulation. Formulation code V7 has shown a promising formula for delivering the drug by which the bioavailability of the drug can be improved, side effects can be reduced, first pass hepatic metabolism of the drug can be avoided and finally the patient compliance can be improved.
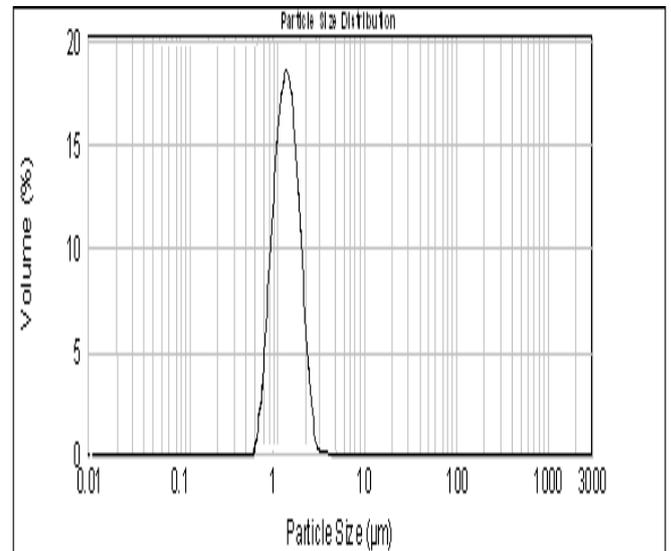
Fig. 13: Particle size distribution graph of optimized V7 formulation batch
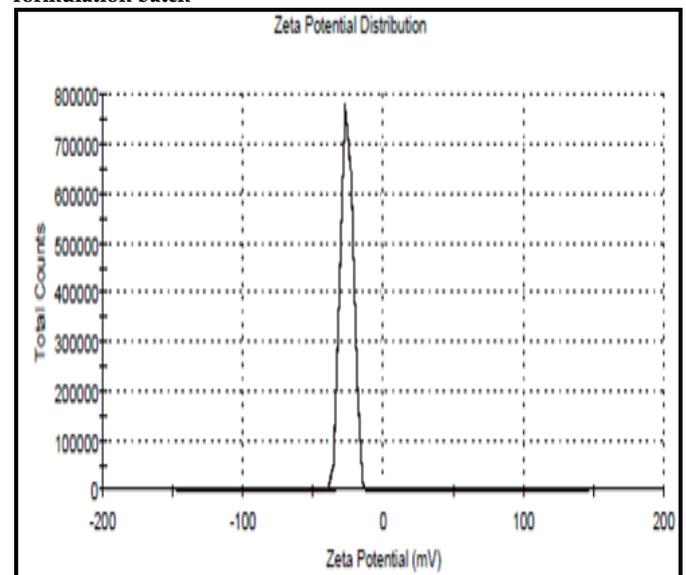
Fig. 14: A typical graph for zeta potential of V7 batch

Table 18: *In-vitro* drug release data of medicated niosome

Time (h)	% Cumulative drug release								
	V1	V2	V3	V4	V5	V6	V7	V8	V9
0	0	0	0	0	0	0	0	0	0
2	4.45	5.02	4.22	5.25	4.45	5.24	**6.45**	3.45	3.56
3	8.34	9.56	7.23	8.64	7.54	8.02	**9.56**	7.55	6.28
4	10.11	12.11	10.25	11.43	10.96	12.89	**15.23**	9.95	9.25
5	14.43	16.34	14.43	14.56	12.53	15.04	**18.25**	13.56	12.43
6	24.21	24.5	22.58	23.41	19.96	20.48	**26.95**	21.11	19.55
7	32.23	34.16	30.23	30.54	29.78	32.11	**34.11**	28.24	26.65
8	39.55	40.11	36.55	37.78	38.57	40.67	**42.56**	42.05	33.43
12	62.45	64.78	60.44	62.65	63.98	65.43	**68.45**	63.45	60.23
18	82.61	83.49	82.54	86.56	85.46	88.9	**92.36**	78.59	75.43
24	90.26	87.67	88.21	91.55	90.23	92.45	**98.55**	85.65	82.25

Fig. 15: *In-vitro* drug release data of medicated niosome formulations
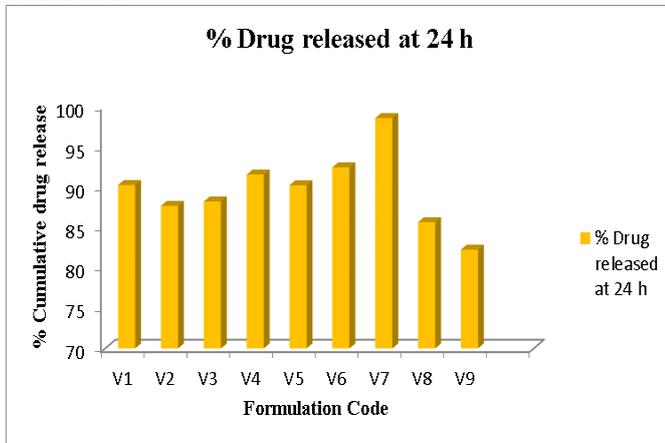
Fig. 16: Bar diagram of % drug release at 24 h for medicated niosome formulations
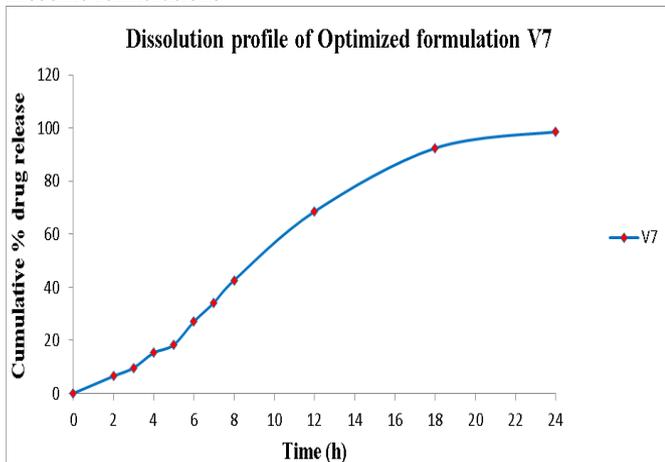
Fig. 17: Dissolution profile of optimized formulation V7

Kinetic modelling and mechanism of drug release

Mathematical models play a vital role in the interpretation of mechanism of drug release from a dosage form. It is an important tool to understand the drug release kinetics of a dosage form. The *in-vitro* drug release profile was applied in different mathematical models i.e. Zero order, First order, Higuchi, Hixson-crowell and Korsmeyer Peppas and was interpreted in the form of graphical presentation and evaluated by correlation coefficient (r2) represented in Table 19. The following plots were made: Time vs. % Cumulative drug release (Zero order model); Time vs. Log % drug remain to be released (First order model), Square root of time vs. % Cumulative drug release (Higuchi model), Time vs. Cubic root of unreleased fraction of drug(Hixson-crowell model) and log time vs. log % cumulative drug release (Korsmeyer peppas model).

Table 19: Kinetic modelling of Valsartan

Formulation code	Zero order kinetics	First order kinetics	Higuchi kinetics	Hixson-Crowell kinetics	Korsmeyer Peppas kinetics
	R2	R2	R2	R2	R2
V1	0.9827	0.9779	0.8853	0.9539	0.9647
V2	0.9730	0.9418	0.8899	0.9718	0.9559
V3	0.9770	0.9728	0.8807	0.9563	0.9714
V4	0.9745	0.9659	0.8800	0.9567	0.9654
V5	0.9768	0.9662	0.8682	0.9698	0.9701
V6	0.9682	0.9606	0.8748	0.9468	0.9642
V7	0.9805	0.9395	0.8929	0.9578	0.9521
V8	0.9759	0.9361	0.8737	0.9702	0.9667
V9	0.9771	0.9730	0.8701	0.9699	0.9755

Zero order model

Table 20: *In-vitro* drug release profile (Time Vs. % Cumulative drug release)

Time (h)	% Cumulative drug release								
	V1	V2	V3	V4	V5	V6	V7	V8	V9
0	0	0	0	0	0	0	0	0	0
2	4.45	5.02	4.22	5.25	4.45	5.24	6.45	3.45	3.56
3	8.34	9.56	7.23	8.64	7.54	8.02	9.56	7.55	6.28
4	10.11	12.11	10.25	11.43	10.96	12.89	15.23	9.95	9.25
5	14.43	16.34	14.43	14.56	12.53	15.04	18.25	13.56	12.43
6	24.21	24.5	22.58	23.41	19.96	20.48	26.95	21.11	19.55
7	32.23	34.16	30.23	30.54	29.78	32.11	34.11	28.24	26.65
8	39.55	40.11	36.55	37.78	38.57	40.67	42.56	42.05	33.43
12	62.45	64.78	60.44	62.65	63.98	65.43	68.45	63.45	60.23
18	82.61	83.49	82.54	86.56	85.46	88.9	92.36	78.59	75.43
24	90.26	87.67	88.21	91.55	90.23	92.45	98.55	85.65	82.25

First order model

Table 21: *In-vitro* drug release profile (Time Vs. Log % drug remain to be released)

Time (h)	Log % drug remain to be released								
	V1	V2	V3	V4	V5	V6	V7	V8	V9
0	2.000	2.000	2.000	2.000	2.000	2.000	2.000	2.000	2.000
2	1.980	1.978	1.981	1.977	1.980	1.977	1.971	1.985	1.984
3	1.962	1.956	1.967	1.961	1.966	1.964	1.956	1.966	1.972
4	1.954	1.944	1.953	1.947	1.950	1.940	1.928	1.954	1.958
5	1.932	1.923	1.932	1.932	1.942	1.929	1.912	1.937	1.942
6	1.880	1.878	1.889	1.884	1.903	1.900	1.864	1.897	1.906
7	1.831	1.818	1.844	1.842	1.846	1.832	1.819	1.856	1.865
8	1.781	1.777	1.802	1.794	1.788	1.773	1.759	1.763	1.823
12	1.575	1.547	1.597	1.572	1.557	1.539	1.499	1.563	1.600
18	1.240	1.218	1.242	1.128	1.163	1.045	0.926	1.331	1.390
24	0.989	1.091	1.072	0.927	0.990	0.878	0.785	1.157	1.249

Higuchi model

Table 22: *In-vitro* drug release profile (Square root of time Vs. % Cumulative drug release)

Square root of time	% Cumulative drug release								
	V1	V2	V3	V4	V5	V6	V7	V8	V9
0.000	0	0	0	0	0	0	0	0	0
1.414	4.45	5.02	4.22	5.25	4.45	5.24	6.45	3.45	3.56
1.732	8.34	9.56	7.23	8.64	7.54	8.02	9.56	7.55	6.28
2.000	10.11	12.11	10.25	11.43	10.96	12.89	15.23	9.95	9.25
2.236	14.43	16.34	14.43	14.56	12.53	15.04	18.25	13.56	12.43
2.449	24.21	24.5	22.58	23.41	19.96	20.48	26.95	21.11	19.55
2.646	32.23	34.16	30.23	30.54	29.78	32.11	34.11	28.24	26.65
2.828	39.55	40.11	36.55	37.78	38.57	40.67	42.56	42.05	33.43
3.464	62.45	64.78	60.44	62.65	63.98	65.43	68.45	63.45	60.23
4.243	82.61	83.49	82.54	86.56	85.46	88.9	92.36	78.59	75.43
4.899	90.26	87.67	88.21	91.55	90.23	92.45	98.55	85.65	82.25

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Hixon crowell model

Table 23: *In-vitro* drug release profile (Time Vs. Cubic root of unreleased fraction of drug)

Time (h)	Cubic root of unreleased fraction of drug								
	V1	V2	V3	V4	V5	V6	V7	V8	V9
0	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
2	0.070	0.079	0.067	0.083	0.070	0.083	0.102	0.054	0.056
3	0.133	0.153	0.115	0.138	0.120	0.128	0.153	0.120	0.100
4	0.162	0.196	0.165	0.184	0.177	0.209	0.249	0.160	0.148
5	0.235	0.268	0.235	0.238	0.203	0.246	0.302	0.220	0.201
6	0.410	0.415	0.380	0.395	0.332	0.342	0.462	0.353	0.325
7	0.565	0.604	0.525	0.531	0.516	0.563	0.603	0.486	0.456
8	0.717	0.73	0.653	0.679	0.696	0.742	0.784	0.772	0.589
12	1.293	1.364	1.235	1.299	1.339	1.384	1.482	1.323	1.229
18	2.051	2.096	2.048	2.264	2.201	2.411	2.572	1.865	1.735
24	2.506	2.332	2.366	2.605	2.504	2.680	2.856	2.212	2.033

Korsmeyer peppas model

Table 24: *In-vitro* drug release profile (Log time Vs. Log % cumulative drug release)

Log time	Log % cumulative drug release								
	V1	V2	V3	V4	V5	V6	V7	V8	V9
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.301	0.648	0.701	0.625	0.720	0.648	0.719	0.810	0.538	0.551
0.477	0.921	0.980	0.859	0.937	0.877	0.904	0.980	0.878	0.798
0.602	1.005	1.083	1.011	1.058	1.040	1.110	1.183	0.998	0.966
0.699	1.159	1.213	1.159	1.163	1.098	1.177	1.261	1.132	1.094
0.778	1.384	1.389	1.354	1.369	1.300	1.311	1.431	1.324	1.291
0.845	1.508	1.534	1.480	1.485	1.474	1.507	1.533	1.451	1.426
0.903	1.597	1.603	1.563	1.577	1.586	1.609	1.629	1.624	1.524
1.079	1.796	1.811	1.781	1.797	1.806	1.816	1.835	1.802	1.780
1.255	1.917	1.922	1.917	1.937	1.932	1.949	1.965	1.895	1.878
1.380	1.955	1.943	1.946	1.962	1.955	1.966	1.994	1.933	1.915
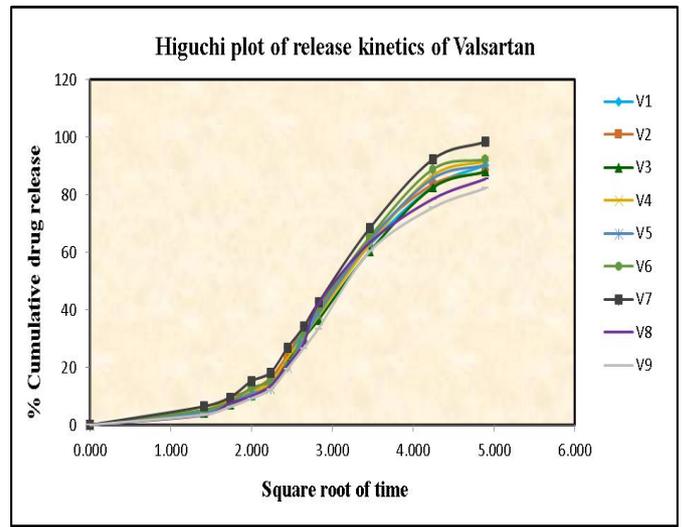
**Fig. 20: Higuchi plot of release kinetics of Valsartan from Niosomes**
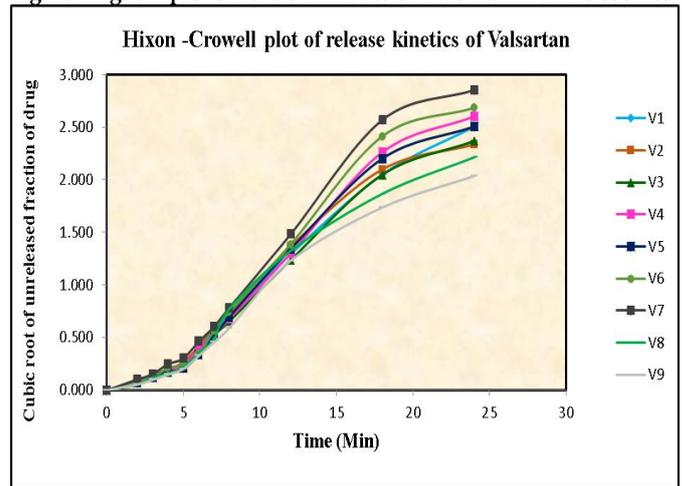
Fig. 21: Hixon crowell plot of release kinetics of Valsartan from Niosomes

**Fig. 18: Zero order plot of release kinetics of Valsartan from Niosomes**

Fig. 22: Korsmeyer peppas plot of release kinetics of Valsartan from Niosomes

Inference

From the above comparison, it was found that Zero order model showed higher degree of correlation coefficient (r^2) 0.9805 for optimized formulation V7 than other models which indicates that the process of constant drug release from dosage form. Hence, the release of drug is in controlled manner.
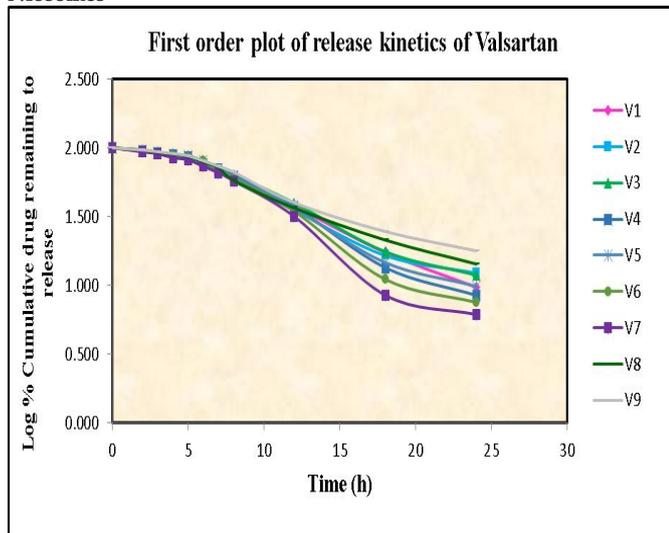
Fig. 19: First order plot of release kinetics of Valsartan from Niosomes

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