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Research Article

Fabrication and *in-vitro* evaluation of valsartan nanosuspensions.

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ABSTRACT
The rationale of the present study was to formulate, characterize and evaluate the nanosuspension of valsartan, a poorly water soluble and low bioavailable drug with intend of recuperating aqueous solubility consequently the dissolution rate. Valsartan nanosuspensions were prepared by solvent evaporation method using different polymers. Materials used in the preparation of nanosuspensions were Tween80, Polaxomer, PVP-K90, PVA, and Ethanol. Different ratio of polymers were used in the formulation of twelve valsartan formulations (VF1 to VF12) The prepared nanosuspensions were characterized by drug- excipient interactions (FTIR) and evaluated for drug content uniformity, particle size analysis, zeta potential, in-vitro drug release and short-term stability. VF11 formulation exhibited 99.86% of drug released within 25 min and exhibited zero order drug release kinetics. Stability studies indicated VF11 formulation exhibited better stability.

KEYWORD
Nanosuspension, solvent evaporation, zeta potential, polymers, particle size.

1. INTRODUCTION

Poor solubility of drug substance has always been a challenging problem faced by pharmaceutical scientists and it is increased now because more than 40% of new chemical entities are poorly water soluble. One of the most persistent problems faced by drugs with poor aqueous solubility is that their oral delivery is frequently associated with implication of low bioavailability and lack of dose proportionality. There are number of technologies like solid dispersion, complexation, co-solvency, use of surfactants, etc., but they lack universal applicability to all drugs. A novel technology that can used to overcome problems associated with this method is nanosuspension, which is based on size reduction mechanism[1].

In the recent past nanosuspensions dragged the attention of formulators and dramatic increase in the formulation of nanosuspensions was observed. Nanosuspension process that can be adopted in the formulation includes precipitation method, high pressure homogenization method, supercritical fluid method, melt emulsification method, emulsion diffusion method etc [2,3]. As precipitation method is endowed with numerous advantages like less production steps, rapid and ease of production, it catches the eye of formulator towards it during their research [4].

In solvent evaporation method, the drug is made to dissolve in the organic solvents like methanol, acetone, acetonitrile or ethyl acetate. The organic solvent is made to evaporate either by continuous stirring or by reducing the pressure. The type of the stabilizer and its concentration, speed of homogenization are the probable factors which decide the size of the particle. In order to reduce the particle size to nano range high shear homogenization or ultrasonication can be executed. Precipitation of the nanosized particles also depends on the degree of supersaturation. Supersaturation level of drug in the solvent can be augmented by the evaporation of the solvent. Formulator has to consider all these factors while designing the nanosuspension by solvent evaporation method [5-8].

Valsartan, angiotensin II receptor antagonist is used in the treatment of hypertension [9]. It helps in improving the symptoms and quality of life in patients having chronic heart failure [10]. Valsartan treatment exhibited no confirmable negative outcome on growth and development and hence it is used safely as an antihypertensive agent even in children whose age is less than 6 years old [11]. Valsartan is rapidly absorbed following oral administration. It has a systemic availability of 25%, which is reduced to about 15% in presence of food. Valsartan has 95% protein binding capacity and is mostly excreted as unchanged form through the bile [12]. It is given in the daily doses of 40–160 mg once a day; the drug available in the systemic circulation is reduced in hepatic impairment, intravascular volume depletion, and renal impairment [13]. The drug is available as conventional tablets 40, 80, and 160 mg commercially. Trials have been carried out to formulate valsartan as a transdermal dosage form to overcome its low oral bioavailability and found satisfactory [14]. Nanosuspension formulations exhibit advantage of bypassing hepatic metabolism.

As nanosuspensions are endowed with bypassing the hepatic metabolism, in this research work we have focused to formulate valsartan as nanosuspension by executing solvent evaporation method.

2. MATERIALS AND METHODS

Valsartan is obtained as a gratis from Dr Reddy's laboratories, Hyderabad. Tween80, Polaxomer, PVP-K90, PVA, Ethanol and other solvents were purchased from Rankem, Mumbai. All the other solvents and reagents used were of pharmaceutical grade.

2.1. Preparation of valsartan Nanosuspension by solvent evaporation technique

Nanosuspension was prepared by the solvent evaporation technique [15,16]. Valsartan was dissolved in acetone at room temperature (organic phase). This was poured into water containing different stabilizers of PVP K90, PVA, Polaxomer, and Tween 80 maintained at room temperature and subsequently stirred on magnetic stirrer which is stirred at rpm 800-1000 for 30 min to allow the volatile solvent to evaporate. Addition of organic solvents by means of a syringe positioned with the needle directly into stabilizer containing water. Organic solvents were left to evaporate off under a slow magnetic stirring of the nanosuspension at room temperature for 1 hour followed by sonication for 1 hour. The composition of valsartan nanosuspension is given in Table 1.

Table 1. Composition of Valsartan nanosuspensions**.**

Ingredients (mg/tablet)	VF 1	VF 2	VF 3	VF 4	VF 5	VF 6	VF 7	VF 8	VF 9	VF 10	VF 11	VF 12
Valsartan	640	640	640	640	640	640	640	640	640	640	640	640
Tween 80 (m1)	0.5	0.5	0.5	0.75	0.75	0.75	1	1	1	1.25	1.25	1.25
PVP K-90	5	--	--	10	--	--	15	--	--	15	--	--
PVA	--	5	--	--	10	--	--	15	--	--	15	--
Polaxomer (mg)	--	--	5	--	--	10	--	--	15	--	--	15
Ethanol (ml)	5	5	5	5	5	5	5	5	5	5	5	5
Water (ml)	40	40	40	40	40	40	40	40	40	40	40	40

2.2. Evaluation of valsartan nanosuspensions [16]

2.2.1. Drug content uniformity

10 ml of each formulation was taken and dissolved in 10 ml isotonic solution and kept overnight. 10 mg (similar as in formulation) of drug was taken and dilution was made to 10µg/ml. The dilutions were filtered and analyzed using UV for their content uniformity. The absorbance of

the formulations was recorded spectrophotometrically at 292nm. The drug content in each formulation was calculated accordingly.

2.2.2. Entrapment efficacy

The freshly prepared nanosuspension was centrifuged at 10,000 rpm for 20 min at 5°C temperature using cooling ultracentrifuge. The amount of unincorporated drug was measured by taking the absorbance of the appropriately diluted 25 ml of supernatant solution at 243 nm using UV spectrophotometer against blank/control nanosuspensions. Drug entrapment efficiency was calculated by subtracting the amount of free drug in the supernatant from the initial amount of drug taken. The experiment was performed in triplicate for each batch and the average was calculated.

The entrapment efficiency (EE %) could be achieved by the following equation:

%Entrapment efficiency= Drug content *100/Drug added in each formulation **---Equation 1**

2.2.3. Zeta potential

The zeta potential is defined as the difference in potential between the surface of the tightly bound layer (shear plane) and the electro-neutral region of the solution. The potential gradually decreases as the distance from the surface increases. The most widely-used theory for calculating zeta potential was developed by Smoluchowski in 1903. The theory is based on electrophoresis and can be expressed as eqn. (2), where (μ) is the electrophoretic mobility, (ε) is the electric permittivity of the liquid, (η) is the viscosity and (ζ) is the zeta potential [17-19].

$\mu=\zeta\varepsilon/\eta$ **---Equation 2**

The zeta potential was determined using Malvern Zetasizer ZS. Zeta potential values will provide information about the stability of the formulation.

2.2.4. Particle size and shape

Average particle size and shape of the formulated nanosuspensions was determined by using Malvern Zetasizer ZS using water as dispersions medium. The sample was scanned 100 times for determination of particle size.

2.2.5. In-vitro drug release study

In-vitro dissolution studies were performed in USP apparatus-II (LAB INDIA DS 8000), employing paddle stirrer at rotation speed of 50 rpm and 200 ml of phosphate buffer pH 6.8 as dissolution medium. Accurately weighed bulk drug and nanosuspensions were dispersed in dissolution medium. The release study is performed at 37 ±0.5^{O}C. Samples of 5 ml are withdrawn at predetermined time intervals and replaced with fresh medium to maintain sink condition. The samples were filtered through 0.22 μm membrane filter disc (Millipore Corporation) and analyzed for valsartan after appropriate dilution by measuring the absorbance at 243 nm.

2.2.6. SEM analysis

The external morphology of the prepared valsartan nanosuspensions was analyzed by a scanning electron microscope (Thermoscientific SU 1510). The formulations were adhered to the brass specimen club using double sided platinum coated electrically conductive adhesive tape for 300 s at 15 Ma.

2.2.7. Stability studies

Short- term stability studies were performed at a temperature of 40 ± 2^{o}C / 75 ± 5% RH over a period of three months (90 days) on the promising nanosuspension of valsartan optimized formulation. Samples were taken at one month interval for drug content estimation. At the end of three month period, dissolution test was also performed to determine the drug release profiles.

3. RESULTS AND DISCUSSION

Valsartan is a BCS class-II drug having low solubility and high permeability. Thus, it is challenging to enhance the solubility of valsartan particles in an aqueous solution. Solvent evaporation technique has been employed to produce nanosuspension of valsartan.

Determination of valsartan λ-max was done in pH 6.8 buffer medium for accurate quantitative assessment of drug dissolution rate. The λ-max was found to be 243 nm, i.e., at its absorption maxima. The linearity was found to be in the range of 5-30 µg/ml in pH 6.8 buffer medium. The regression value was closer to 1 indicating the method obeyed Beer-lamberts' law.

Solubility studies for valsartan was carried out at 25°C using 0.1N HCL, pH 6.8 phosphate buffer and purified water. Valsartan exhibited highest solubility in pH 6.8 phosphate buffer when compared to other buffer solutions. The solubility results were depicted in Figure 1.

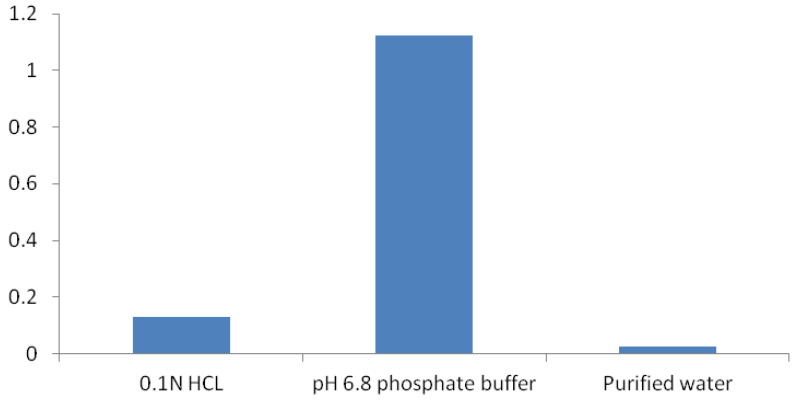

Fig. 1. Solubility of Valsartan.

Compatibility studies were performed using IR spectrophotometer. The FTIR spectrum of pure drug and physical mixture of drug and excipients were studied as depicted in Figure 2. The results endowed that no significant interaction had occurred between drug and excipients.

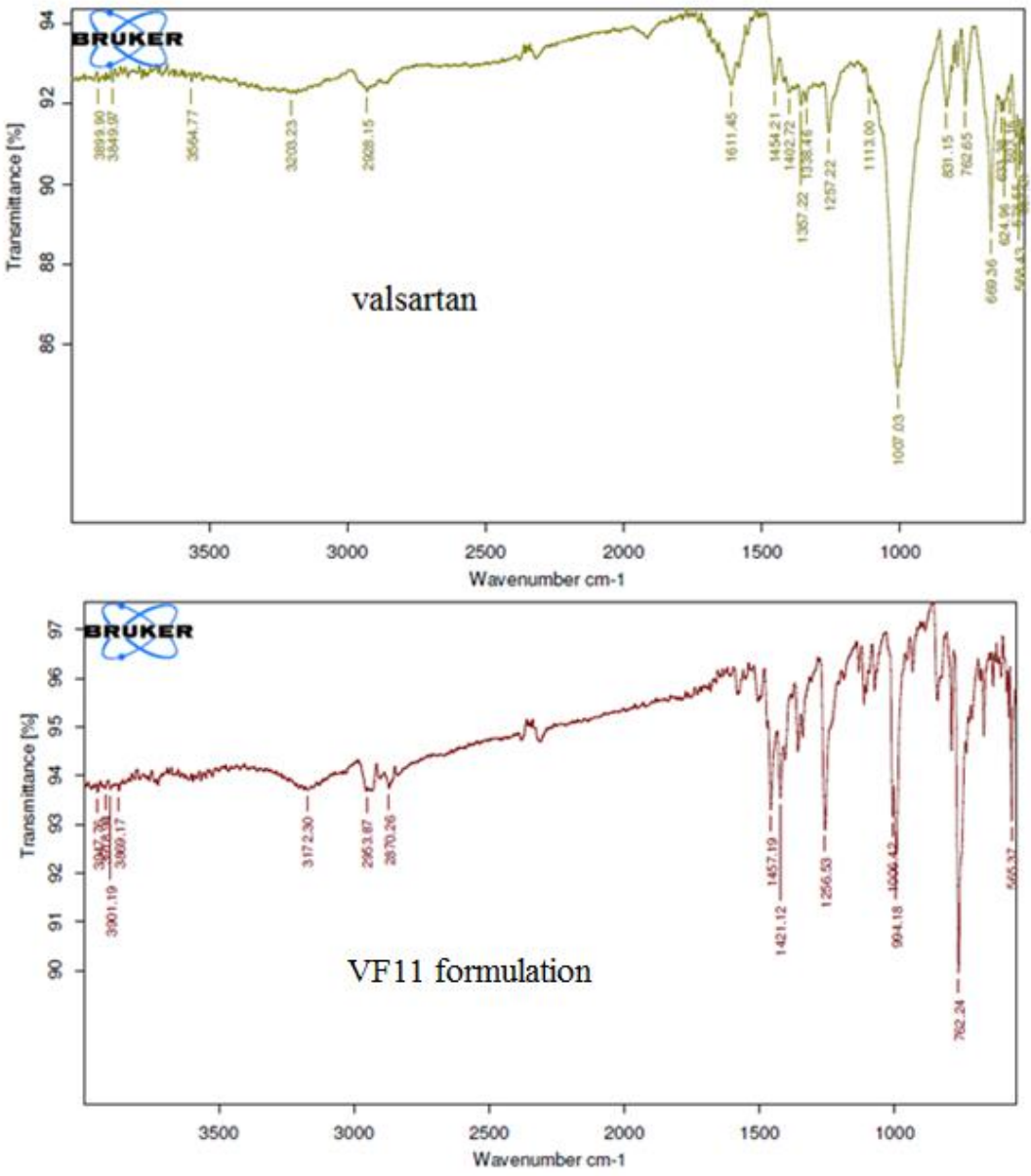

Fig. 2. FTIR of valsartan and valsartan nanosuspenion.

The drug content of the formulated valsartan nanosuspension was found in the range of 78.23 to 96.45 % as given in Table 2.

Table 2. Drug content of formulated nanosuspension.

Formulation code	Mean % drug content
VF1	78.23
VF2	80.14

VF3	82.24
VF4	84.22
VF5	89.64
VF6	92.31
VF7	88.42
VF8	94.76
VF9	91.45
VF10	92.86
VF11	96.12
VF12	95.02

The entrapment efficacy of the formulated valsartan nanosuspension was found to be in the range of 85.70±0.99% to 94.12±0.47% respectively as given in Table 3.

Table 3. Entrapment efficiency of valsartan nanosuspensions.

Formulation code	**Mean % entrapment efficiency**
VF1	82.16
VF2	86.14
VF3	88.48
VF4	91.36
VF5	89.62
VF6	90.48
VF7	92.38
VF8	93.92
VF9	91.38
VF10	92.12
VF11	94.86
VF12	91.25

The *in-vitro* dissolution data of all the designed formulations are shown and dissolution profiles depicted in figure 3. From the results it was observed that VF11 had exhibited good release. Thus it is selected as the better formulation for further characterization.

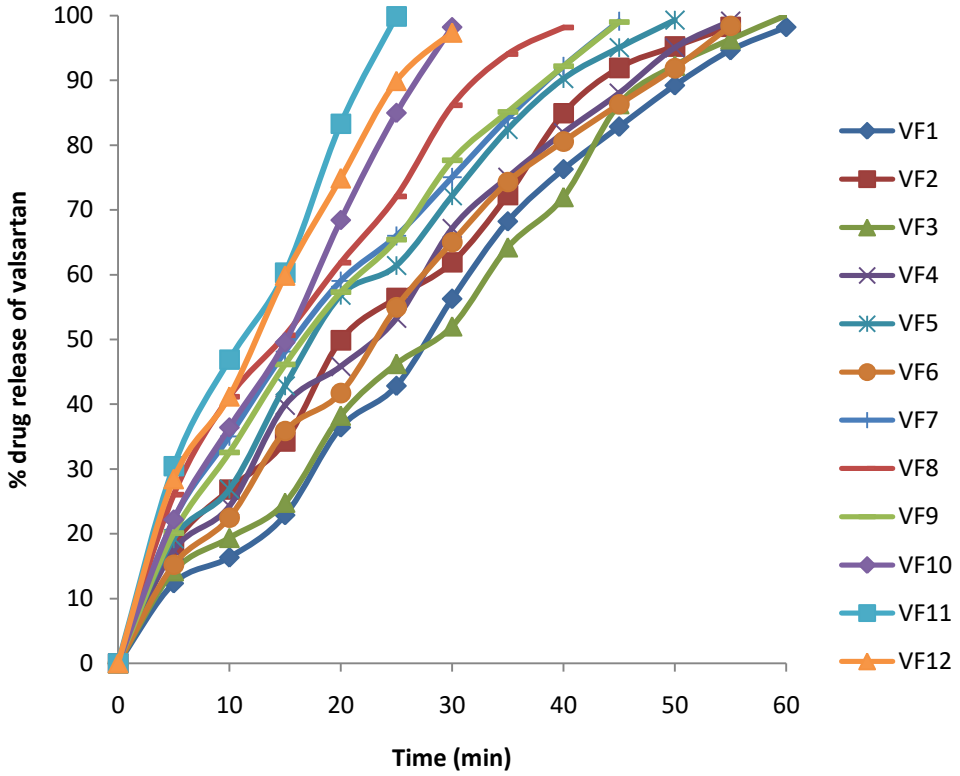

Fig. 3. *In-Vitro* Drug Release Profiles of valsartan nanosuspensions VF1 - VF12.

In-vitro drug release data of all valsartan nanosuspension formulations was subjected to goodness of fit test by linear regression analysis according to zero order, first order kinetics and according to equations of drug release. The results of linear regression analysis including regression coefficients from the above data it is evident that the optimized formulation (VF11) follows zero-order release kinetics. SEM analysis carried out for selected VF11 formulation was depicted in figure 3. From the report it was observed that the particles were uniform in size and is of nano range.

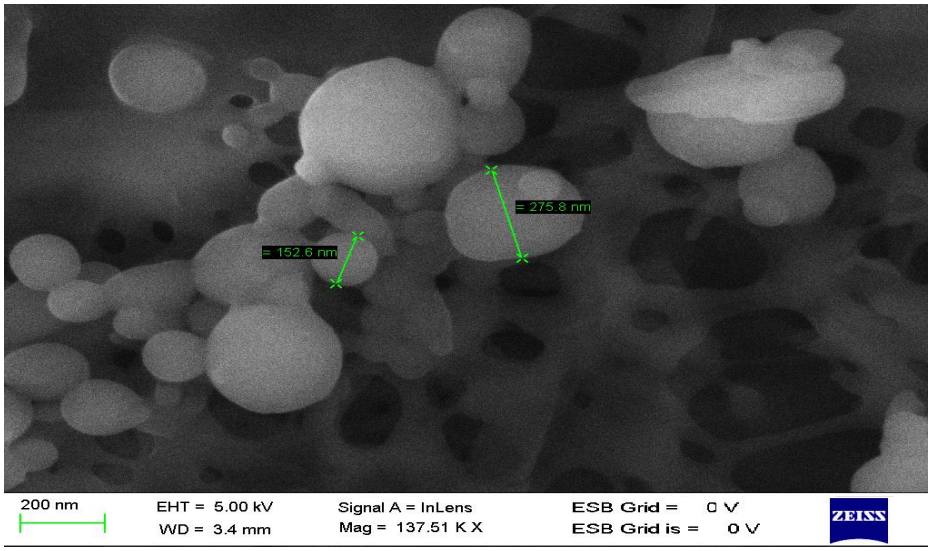

Fig. 4. SEM analysis of valsartan best formulation (VF11).

Particle size distribution was carried out for VF11 formulation which clearly indicated that maximum particles are at a range of 118 nm as shown in figure 4.

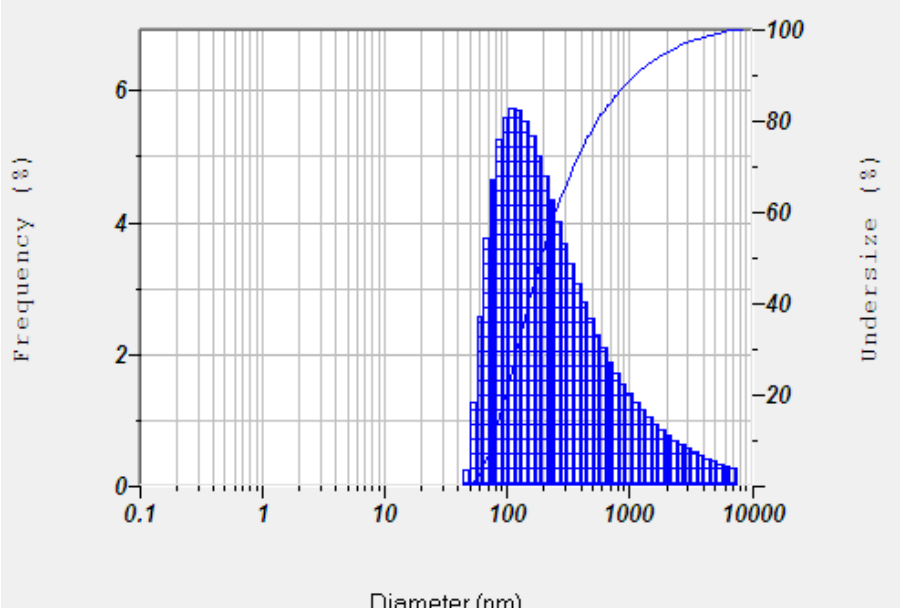

Fig. 5. Particle size Analysis for VF11 formulation.

Zeta potential value for the optimized formulation (VF11) was found to be within the acceptable limits of -10 mV.

From the stability studies of optimized formulation VF11 which has been carried out for about 90 days, by comparing the results it was observed that there is no significant change in the dissolution profile of optimized formulation on storage which indicates that it passes the stability studies.

4. CONCLUSION

Oral Nanosuspension of valsartan can be prepared by solvent evaporation method using combinations of polymers Tween 80, Polaxomer, PVP-K90, PVA, Ethanol and quantity sufficient of distilled water. The melting point of Valsartan was found to be in range of 110°C which was determined by capillary method. Saturation solubility was carried out at 25°C using 0.1N HCL, 6.8 phosphate buffer, and purified water and valsartan exhibited highest solubility in 6.8 phosphate buffer. From the drug excipient compatibility studies we observe that there are no interactions between the pure drug and excipients. The percentage of drug content of formulations was found to be 78.23%-96.12% respectively. The entrapment efficacy of formulations VF1-VF12 was found to be 82.16-94.86% respectively. The zeta potential value for the optimized formulation (VF11) was found to be within the acceptable limits. Average particle size of nanosuspension of optimized formulations (VF11) was found to be 118nm. From the *in vitro* studies we can say that formulation VF11 shows best drug release of 99.86% within 25 minutes where as all the other formulations takes about 45-60 minutes to release the drug. Thus Valsartan nanosuspensions can be a novel promising systems for controlled released dosage form which is gaining importance because of ease of manufacturing and diversified applications. The present trend of pharmaceutical research lies in the usage of biodegradable polymer because of its availability and low toxicity.

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