Preparation and *in vitro* & *in vivo* characterization of valsartan loaded eudragit nanoparticles

M.Bharathi[a] , Sarat Chandra Prasad M[b] , R. Latha Eswari[a], S.Wasim Raja[d], Ravi Teja Allena[b], S.Brito Raj*[c], K. Bhaskar Reddy[c]

[a]*Department of Pharmaceutics, Periyar College of Pharmaceutical Sciences for Girls, Tiruchirappalli–620 021, Tamil Nadu, India.*
[b]*Department of Pharmaceutics, J.S.S College of Pharmacy, JSS University, Mysore – 570015, Karnataka, India.*
[c]*Department of Pharmaceutics, Sri Venkateswara College of Pharmacy, R.V. S. Nagar, Chittoor–517127, Andhra Pradesh, India.*
[d]*Department of Clinical Pharmacy, Sri Venkateswara College of Pharmacy, R.V. S. Nagar, Chittoor–517127, Andhra Pradesh, Indi*a.

_____

ABSTRACT

The aim of the present work was to prepare and investigate the valsartan nanoparticles. nanoparticles were prepared by nanoprecipitation method and the various formulations were prepared by optimizing. The prepared nanoparticles were characterized by FTIR, DSC, SEM, particle size analysis, In vitro diffusion and in vivo studies are been performed. The particle sizes of the prepared nanoparticles were ranging from 175 to 232 nm. From three formulations, F2 formulation showed best release of 60.38 % at the end of 24th h. In vivo studies revealed that in case of free drug, 40.9 mcg/ml drug of maximum dose was recovered but in case of nanoparticles the dose recovered in serum was 16.02 mcg/ml after 6th h. The formulation stored at 4[o] ± 1 [o]C was more stable compared to the other temperatures. These studies suggest that the feasibility of formulating valsartan loaded Eudragit L 100 nanoparticles for the treatment of hypertension by enhancing the bioavailability.

Keywords: *In vitro* release studies, *In vivo* studies, Nanoparticles, Particle size, SEM, Valsartan.
_____

INTRODUCTION

More than 72 Million Americans have hypertension, and the majority of these persons have essential hypertension. The most common cause of secondary hypertension is renal vascular hypertension, of which renal artery stenosis is the leading pathology. Upto 5 percent of all occurrences of hypertension are caused by renal artery stenosis, equating to as many as 3.5-4 million occurrences in the United States. Detecting renal artery stenosis is particularly important for ensuring that this potentially curable form of hypertension is identified and treated properly. Duplex Doppler ultrasonography is a good screening test in many patients, but it has limitations in larger persons and can overlook small accessory arteries [1].

[2] The development of angiotensin II receptor antagonists is one of the latest advances in the pharmacological treatment of hypertension. [3] Valsartan peak plasma levels reaches maximum at range of 2 to 4 h after oral administration. [4 and 5] The oral dosage of valsartan is 80 to 320 mg once daily. [6] Valsartan Plasma concentration and AUC will reduce due food intake by 40 to 50 %. [7] The elimination half-life is about 6 h. for the poor soluble drugs drug through oral delivery is one of the major challenges to formulation.[8] Formulation of

polymeric nanoparticles of Valsartan loaded Eudragit can be used for the treatment of hypertension by reducing the dose and frequency of administration of drug.

[9] Reported the solubility of valsartan is poor in water and its low dissolution rate in aqueous G.I.T fluid often leads to insufficient bioavailability. [10] Has formulated Carvedilol loaded Eudragit E 100 Nanoparticles by using nanoprecipitation method using polymeric stabilizer Poloxamer 407. [11] Has formulated and evaluated double coated poly (butyl cyanoacrylate) nanoparticles for brain targeting of D-Kyotrorphin via oral administration. [12] Observed the presence of Tween 80 nanoparticles, particle size enlargement, but relevantly the surfactant coating layer led to a marked zeta potential reduction. [13] Has prepared polymethacrylic acid nanoparticles containing Lamivudine in different drug to polymer ratio by nanoprecipitation method. [14] Has reported aliskiren, a direct renin inhibitor improves no bioavailability and protects against spontaneous atherosclerotic changes. [15] Developed Eudragit nanoparticles revealed a decreased t_{min} and enhanced bioavailability and sustained activity. [16] Has reported that nanoparticles made of anionic Eudragit (L-100, L-100-55 and S-100) were obtained by a novel pH nanoprecipitation method. In order to stabilize the nanosuspensions, different stabilizers are added. [17] Has studied to improve the stability of cloricromene in ophthalmic formulation using Eudragit. It improves the shelf life and bioavailability of the drug after ophthalmic application. [18] Has studied the HPLC method for the determination of Valsartan in human plasma. It was performed on an octadecyl silica columns, mobile phase used was acetonitrile and dihydrogen potassium phosphate. [19] Observed ketoprofen crystallization when the amount of drug in Eudragit L nanoparticles was more than 33 %. [20] Has reported Angiotensin AT2 receptors directly stimulate renal nitric oxide in Bradykinin b2 – receptor-null mice. [21] Has prepared Eudragit loaded Ibuprofen nanoparticles by emulsion polymerization technique. The drug loading capacity was increased by increasing the polymer concentration.

Hence in present study we investigated the optimizing parameters like drug, surfactant ratio, stirring time and stirring speed on entrapment efficiency. Final optimized valsartan nanoparticle formulations were evaluated for characterization, *In vitro* studies were carried by diffusion and from the final optimized formulation one of the best formulation is evaluated for *in vivo studies* with rabbit as an animal model (Sprague dawley) and blood samples were estimated by the HPLC and pharmacokinetics parameters are calculated.

MATERIALS AND METHODS

Valsartan (MW 435.52 g/mol) obtained from Microlabs, Hosur, India. Eudragit L-100 (MW ≥ 100,000) obtained from ROHM GMBH Chemiche fabric, Darmstadt. Tween 80 (MW 1310 g/mol) was purchased from Kemphasol, Bombay, India. Acetone (MW 58) and Ethanol were purchased from Fischer chemicals ltd Chennai, India. Disodium hydrogen phosphate was purchased from Nice chemicals Pvt Ltd Chennai, India. Potassium dihydrogen phosphate was obtained from Hi-pure fine chem. industries Pvt Ltd Cochin, India. Membrane filter 0.2 μm was purchased from Sigma Aldrich cheme, Germany. Respectively all chemicals were of analytical grade.

3.1 Preparation of Eudragit nanoparticles
Prepared drug loaded Eudragit L 100 nanoparticles by nanoprecipitation method. Valsartan was first dissolved in small amount of ethanol (2 ml) and polymer was dissolved in acetone. Finally both the polymer and drug were mixed (8 ml). The organic phase was added at a constant flow rate (0.3 ml/min) into 20 ml of aqueous phase containing 2 % tween 80 under magnetic stirring. The organic solvent was then evaporated under room temperature. The suspension was filtered by 0.2 μm membrane and centrifuged under 9000 rpm for 30 min at 5 ^{o}C and nanosuspension was obtained[22, 23].

During the nanoparticle preparation the process variables and formulation variables like the drug, surfactant ratio, stirring rate and stirring time were optimized for getting small spherical shaped nanoparticles with maximum entrapment efficiency and for better bioavailability. The method was first optimized for the choice of drug, surfactant, stirring rate and time. This was used to investigate the effect of entrapment ratio. Finally, Drug entrapment was used to investigate the effect of drug: surfactant ratio, stirring rate and stirring time on mean entrapment efficiency (% EE).

3.2 Characterization of Eudragit nanoparticles
The prepared Eudragit nanoparticles using valsartan were characterized for various characters such as particle size distribution, surface morphology, drug entrapment efficiency, drug loading and DSC studies.

3.3 FTIR compatibility study
The VAL was subjected to FT-IR (FT-IR- 8400, Shimadzu Co., Japan) analysis by pressed pellet method, an approximately minimum quantity of samples was thoroughly blended with adequate quantity of IR grade KBr in

517

_____

mortar. The mix was then made into KBr pellets by hydraulic press. The samples were then analysed in a double beam IR spectrometer using KBr film as negative control (blank). The scanning range was 4000 - 400 cm^{-1} and resolution was 16 cm^{-}. Spectra of the valsartan and the drug with Eudragit L-100 were compared for the compatibility.

3.4 Differential Scanning Calorimetry
This was performed by using (Shimadzu-DSC –TA 60). The samples were placed in aluminium pans and were crimped, followed by heating under nitrogen flow at a scanning rate of 5 ^{o}C / min from 25 ^{o}C to 200 ^{o}C. Empty pan was used as reference. The heat flow as a function of temperature was measured for both drug-polymer mixture and polymer [24].

3.5 Scanning electron microscopy (SEM)
The morphology and surface of nanoparticle were observed using scanning electron microscopy (JEOL JSM –6400, Jeol ltd. Tokyo Japan). The samples of freeze dried nanoparticle were dispersed in water, air dried over metallic studs and coated with platinum [25, 26].

3.6 Particle size distribution
The selected best VAL nanoparticulate suspension (F2) was subjected to laser particle counting method using nanosizer (Microtrac S3500 SI) for characterization of its size distribution. The sample was injected into the sample delivery and controlling chamber. Then, suitable solvent was pumped through the chamber. Now a beam of laser light was allowed to fall on the sample cell. After required number of runs, they were directed towards the detector. From this the particle size range and the average mean particle size of the formulation can be studied [27].

3.7 Entrapment efficiency
A redispersed suspension of nanoparticles in phosphate buffer solution pH 6.8 was used for determination of drug content and entrapment efficiency in the nanoparticle. To 1 ml of VAL nanoparticle suspension, 1 ml of Acetonitrile was added preferentially to precipitate the polymer. 1 ml of aqueous sodium hydroxide solution was added to the above solution and centrifuged at 9000 rpm in cooling centrifuge at 15 ^{o}C for 30 min. The clear supernatant fluid was removed and analysed spectrophotometrically at 250 nm.

3.8 In vitro release studies
The *in vitro* release of VAL from the nanoparticles was studied by simple diffusion cell apparatus. The diffusion cell apparatus consists of a glass tube with an inner diameter of 2.5 cm, open at both ends. One end of the tube was tied with dialysis membrane, which serves as donor compartment. 6 ml of formulation was taken in a diffusion cell and placed in 200 ml phosphate buffer of pH 6.8. The medium was stirred by using magnetic stirrer and temperature was maintained at 37 ^{o}C ± 0.5 ^{o}C, for nanoparticle formulations samples were withdrawn at various time intervals like 0, 1, 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22 and 24 h. The concentration of VAL in samples was determined by analysing spectrophotometrically at 250 nm.

3.9 In vivo studies
The study was conducted after getting approval from the ethics committee. The selected formulation F-2 was carried out by using rabbit as an animal model (Sprague dawley). The healthy rabbits of either sex were selected. They were fasted overnight before administration of dose. The animals were weighed and numbered and they were divided into 4 groups of each containing 6 rabbits. To the first group aqueous solution of VAL was administered. The dose given was 5 mg drug solution and it was administered orally. Nanoparticles loaded with VAL and polymer were administered to second, third and fourth groups.

3.10 Stability studies
It generates information on which proposal for shelf life of drug or dosage form and their recommended storage conditions. The prepared formulations were tested for stability by storing them at 4° ± 1 ^{o}C in refrigerator, 25° ± 2 ^{o}C, 40° ± 2 ^{o}C and 70 % RH in stability testing chamber. Formulations were stored in amber coloured glass vials at 4° ± 1 ^{o}C, 25° ± 2 ^{o}C and 40° ± 2 ^{o}C with 70 % RH for 3 months. After 1st, 2nd and 3rd month they were evaluated [28, 29, 30].

3.11 Release kinetics
In order to understand the mechanism and kinetics of drug release, the drug release data of the *in vitro* dissolution study was analysed with various kinetic models like zero order, first order, Higuchi's, Korsmeyer-Peppa's and Coefficient of correlation (R^2) values. [31, 32, 33] Data obtained from *in vitro* release studies was fitted to various kinetic equations.

First order model can be expressed as,

$$Mt/M\alpha = 1-e^{-kt} \text{-----------} 1$$

Zero order release would be predicted by the following equation

$$At = A_0 - K_0 t \text{--------------} 2$$

Drug release from the matrix devices by diffusion has been described by following Higuchi's classical diffusion equation

$$Q = [D\varepsilon / \tau (2 A - \varepsilon C_s) C_{st}]^{\frac{1}{2}} \text{---} 3$$

3.12 Statistical analysis

Statistical analysis was carried out by using Graph Pad Prism 5 for windows, Version 5.04, USA. Statistical comparisons were performed by means of one-way ANOVA, followed by Newman-Keuls Multiple Comparison Test when more than two groups were compared. A P value of < 0.05 was considered statistically significant.

RESULTS AND DISCUSSION

3.1 Optimisation of formulation

The nanoparticles were prepared by nanoprecipitation method. The nanoparticles were prepared by optimizing various parameters such as drug concentration, polymer concentration, surfactant ratio, stirring time, stirring speed etc.

3.2 Effect of drug on entrapment efficiency

The drug concentration was varied from 5 mg, 7.5 mg and 10 mg in Eudragit nanoparticles. Higher entrapment efficiency and desired particles size was obtained by using (n) mg of drug. On further increase there was no change in the entrapment efficiency which could be due to the saturation of the polymer dispersion. So the optimized drug concentration was found to be 10 mg. The results are shown in table 1 and figure 1.

Table 1. Optimization of drug

Drug (mg)	Polymer (mg)	Surfactant (%)	Stirring time (h)	Entrapment efficiency (%)
5	50	2	5	61.13 ± 1.18
7.5	50	2	5	69.18 ± 1.19
10	50	2	5	78.79 ± 1.16

Optimum parameter, the values are mean ± SD (n =3)

3.3 Effect of surfactant on entrapment efficiency

The surfactant ratio i.e. Tween 80 has an effect on the nanoparticles. The surfactant concentration in the continuous phase, providing a thin protective layer around the droplets and hence reducing in their coalescence. When the concentration of the surfactant is too low, then the aggregation of the polymer droplets occurred. By increasing the concentration of the surfactant from 1 %, 1.5 %, 2 %, 1 - 2 % the entrapment efficiency was increased and the particle size also gets increased. The encapsulation efficiency also was increased from 59.57 % - 78.65 %. The optimum Tween 80 concentration was found to be 2 % for getting the higher entrapment efficiency and desired particle size range. The results are shown in table 2 and figure 2.

Table 2. Optimization of surfactant

Drug (mg)	Polymer (mg)	Surfactant (%)	Entrapment efficiency (%)
10	25	1	59.57 ± 1.13
		1.5	65.79 ± 1.12
		2	69.75 ± 1.06
10	50	1	64.52 ± 1.03
		1.5	70.83 ± 1.08
		2	78.65 ± 1.16
10	75	1	56.89 ± 1.13
		1.5	60.71 ± 1.06
		2	65.65 ± 1.03

Optimum parameter, the values are mean ± SD (n =3)

_____

3.4 Effect of stirring time on entrapment efficiency

The stirring time was also optimized to get small spherical shaped nanoparticles with high entrapment efficiency. It was found that by increasing the stirring time for 3 h, 5 h and 7 h, the entrapment efficiency was increased from 59.72 % - 78.65 % respectively and then it decreased to 55.62 % at 7 h. Finally the stirring time optimized to 5 h and the results are shown in table 3 and figure 3
.

Table 3. Optimization of stirring time

Drug (mg)	Polymer (mg)	Surfactant (%)	Stirring time (h)	Entrapment efficiency (%)
10	25	2	3	60.70 ± 1.11
			5	69.75 ± 1.06
			7	51.52 ± 1.21
10	50	2	3	68.76 ± 1.36
			5	78.65 ± 1.22
			7	60.85 ± 1.13
10	75	2	3	59.72 ± 1.21
			5	65.65 ± 1.06
			7	55.62 ± 1.26

Optimum parameter, the values are mean ± SD (n =3)

3.5 Effect of stirring speed on entrapment efficiency

By increasing the stirring speed from 400 rpm, 500 rpm and 750 rpm, the entrapment efficiency and desired particle size was also increased and then it decreased with the increase in the speed of 750 rpm. Desired particle size was in the range of 208 nm and higher % of 78.65 % for the optimized formulation of Valsartan loaded Eudragit Nanoparticles. F1, F2 & F3 were using 24 mg, 50 mg, 75 mg of Eudragit respectively and it contained 10 mg of drug, 2 % Tween 80 for 5 h at 750 rpm. The results are shown in figure 4 and table 4.

Table 4. Optimization of stirring speed

Drug (mg)	Polymer (mg)	Surfactant (%)	Stirring speed (rpm)	Entrapment efficiency (%)
10	25	2	400	55.75 ± 1.03
			500	60.70 ± 1.02
			750	69.75 ± 1.07
10	50	2	400	63.25 ± 1.23
			500	68.76 ± 1.02
			750	78.65 ± 1.16
10	75	2	400	56.89 ± 1.13
			500	59.72 ± 1.21
			750	65.65 ± 1.07

Optimum parameter, the values are mean ± SD (n =3)

3.6 Final optimised formulation

By the optimisation results obtained. F1, F2 & F3 were using 24 mg, 50 mg, and 75 mg of Eudragit respectively. It contained 10 mg of drug, 2% Tween 80 and stirred for 5 h at 750 rpm. Final optimized formulation with all the parameters was shown in table 5.

Table 5. Final optimized formulation of drug loaded Eudragit L 100 nanoparticles

Formulation code	Drug (mg)	Polymer (mg)	Surfactant (%)	Stirring Time (h)	Stirring speed (rpm)
F1	10	25	2	5	750
F2	10	50	2	5	750
F3	10	75	2	5	750

*3.7 FTIR Spectrum of Valsartan and Eudragit L 100*

IR spectrum of Valsartan showed peaks at 3431 cm^{-1} (CH-X stretch), 2963 cm^{-1} (-CH aromatic), 2737 cm^{-1} (-CH2-), 1600 cm^{-1} (O=C-) and broad peak in the region of 1729 cm^{-1} due to >C=N. Eudragit L-100 showed typical peaks at 3497 cm^{-1}, 2988 cm^{-1} and 1721 cm^{-1} for OH stretch, CH-X and C=O respectively while in IR spectrum of Valsartan with Eudragit L 100 nanoparticles, the O-H group disappeared due to possible cross linking of Eudragit with drug *via* CH-X and keto functionalities and the results are shown in Figure 5. Formulation peaks at 3492 cm^{-1} (CH-X stretch from Valsartan) appeared in the IR spectrum of nanoparticles. (-CH aromatic) peak at 2960.55 cm^{-1}, (-CH2-) at 2606.88 cm^{-1}, 0=C- at 1725 cm^{-1}and (> C=N) at 1604 cm^{-1}. This study confirms entrapment of drug with polymer and the formulations shows the chemical compatibility is not present.

_____

Figure 1. Fourier Transform Infra-red spectrum for valsartan and physical mixture
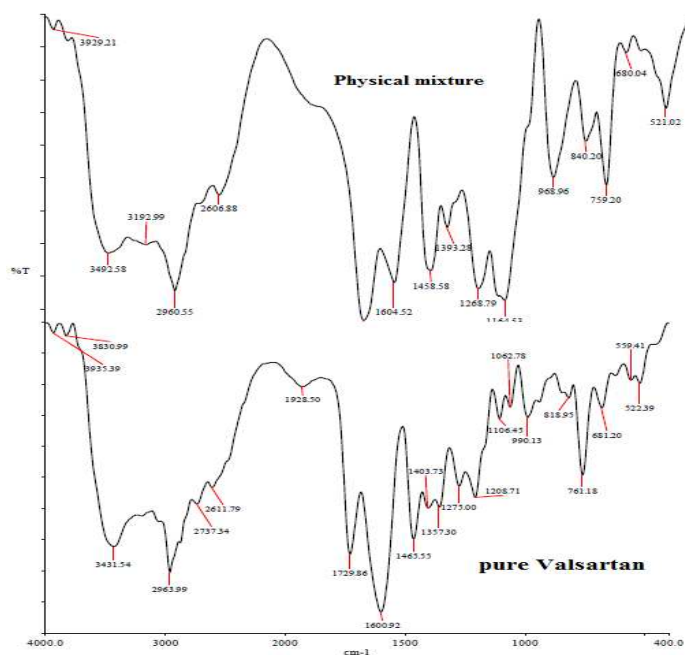
3.8 Differential scanning calorimetry (DSC)

The DSC curve of VAL showed characteristic peaks at 102.81 ^{o}C. The thermogram of Valsartan and Eudragit L 100 mixture exhibited same characteristic peaks of Valsartan at 103.43 ^{o}C. The results of the thermogram suggested that there was no physical interaction between Valsartan and Eudragit L 100. Thermogram was shown in figure 6.

Figure 2. Differential scanning calorimetry (DSC) thermogram of valsartan (a) Eudragit L – 100 (b)
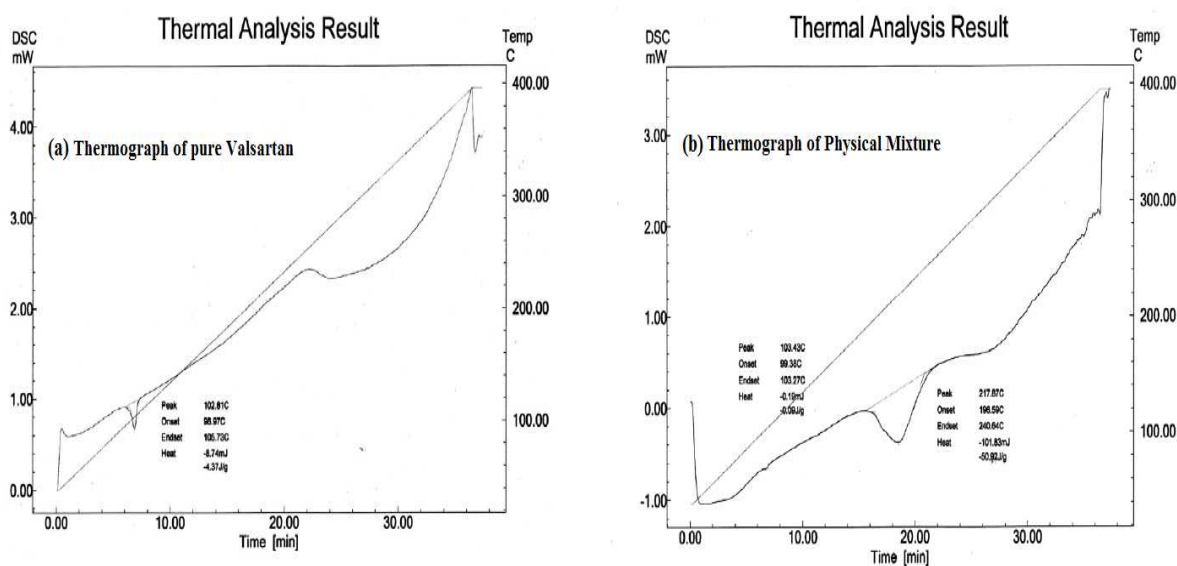
3.9 Scanning electron microscopy

The prepared nanoparticles were characterized by the surface morphology and particle size distribution. The surface morphology was performed by SEM analysis which showed the small spherical shaped, discrete particles without aggregation and smooth texture in surface morphology and the results were shown in figure 7

3.10 Particle size analysis

The particle size distribution was studied by laser counting method. It revealed that the particle size range was around 200 nm, average particle size of F1 formulation was 175 ± 1.06 nm, average particle size of F2 formulation was 205 ± 1.09 nm and average particle size of F3 formulation was 232 ± 1.03 nm and the results are shown in figure. 8 and table 6.

521

_____

Figure 3. SEM photomicrographs (a) Plain Eudragit L 100 nanoparticles & (b) drug loaded Eudragit L 100 nanoparticles
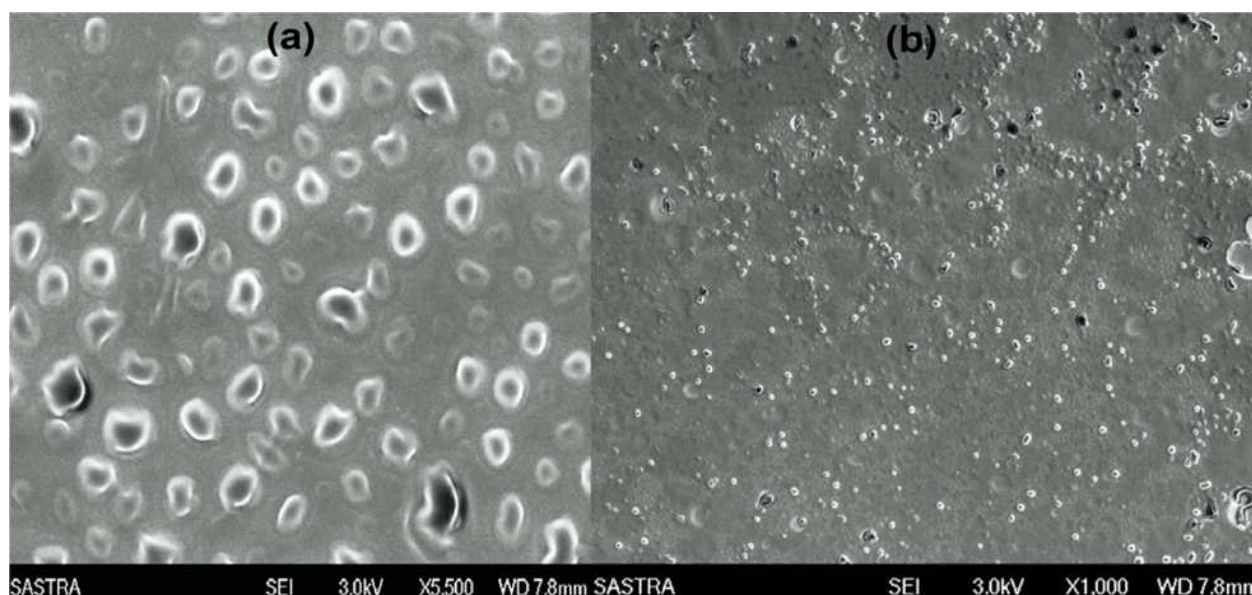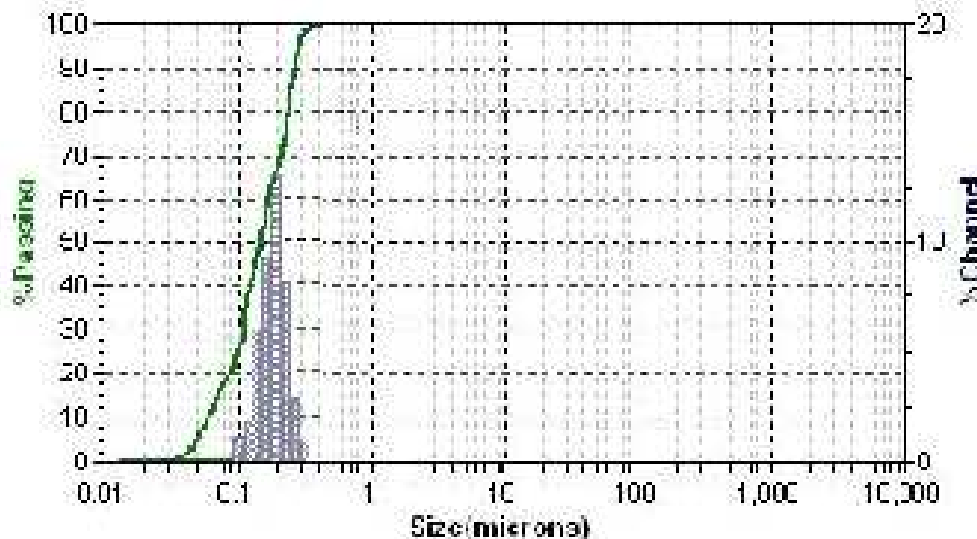

Table 6. Drug loading and particle size of prepared nanoparticles

| Formulation code	Entrapment efficiency (%)	Drug loading (%)	Particle size (nm)
F1	69.75 ± 1.06	29.06 ± 1.13	175 ± 1.06
F2	78.65 ± 1.13	38.5 ± 1.06	205 ± 1.09
F3	65.65 ± 1.06	33.87 ± 1.20	232 ± 1.03

Figure 4. Particle size analysis for F– 2 formulation

3.11Entrapment efficiency and Drug loading
Best entrapment was observed in the F2 formulation i.e. about 78.65 ± 1.13 % and drug loading was about 38.5 ± 1.06 % and the results were shown in table 6.

3.12In vitro drug release profile
The *in vitro* release profiles of VAL loaded Eudragit nanoparticles were performed for F1, F2 and F3. The release behaviour of drug from the polymer matrix followed sustained release. The formulations F1, F2 and F3 showed the release of 65.83 %, 74.17 % and 60.38 % respectively in pH 6.8 at the end of 24th h. There was only negligible amount of drug released at pH 1.2 because drug VAL was acidic in nature so the drug was absorbed only in the pH > 5. From this release profile, the formulation F3 has best release when compared to other formulations. The cumulative % release of 3 formulations and the comparative study were shown in the figure 9.

_____

Figure 5. *In vitro* drug release profile of Eudragit L 100 nanoparticles at pH 6.8
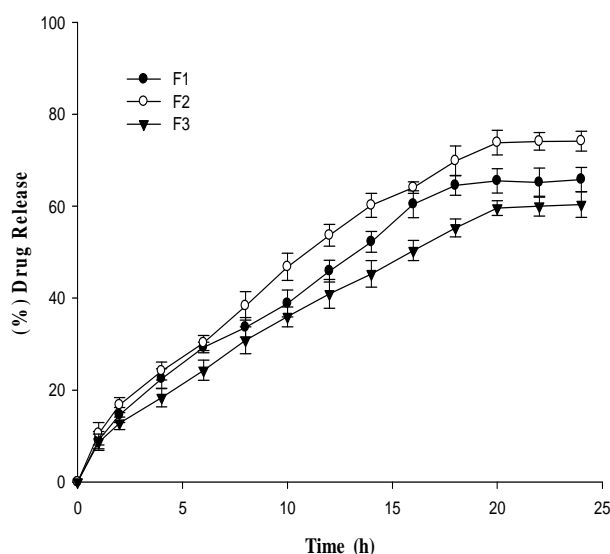
Results were statistically analysed by one-way analysis of variance (ANOVA) with post-test (Newman-Keuls Multiple Comparison Test) at different time intervals from 0 h to 24 h. Statistically significant differences between *in vitro* drug release of formulations were defined as $P < 0.05$. Calculations were performed with Graph Pad Prism software program. The *in vitro* release data of formulations F1, F2 and F3 were compared by one-way ANOVA (Newman-Keuls multiple comparison) test. The *in vitro* release in VAL (pH 6.8) from F2 after 24 h was found to be significant ($P < 0.05$). Significant differences ($p < 0.05$) were also observed for the amount of drug released after 24 h.

3.13In vivo studies
The best formulation F2 from the *in vitro* diffusion studies was selected for performing *in vivo* studies. It was concluded that the C_{max} was decreased; t_{max} and MRT were increased. From the time vs concentration of the drug profile of free drug Valsartan and formulation F-2, the pharmacokinetic parameters were derived.

3.14In vivo pharmacokinetic study
The estimation was carried out on ODS, Phenomonex, C-18 (250 x 4.6 mm, 5 μ) column and absorbance was recorded at 250 nm by using UV detector. Blood was collected from ear portal vein and taken in a centrifuge tube containing EDTA (anticoagulant) and centrifuged at 2000 rpm for 15 min. Supernatant was collected and acetonitrile (1 mg/ml) was added to precipitate the proteins. The precipitated proteins were settled by centrifugation at 2000 rpm for 15 min and supernatant was collected. 1 ml of collected supernatant was filtered through 0.45 µm membrane filter in 10 ml volumetric flask. Volume was made up with 10 ml phosphate buffer solution of pH 6.8 and the absorbance was recorded against blank prepared from serum of Rabbit, to which the drug or formulation was not administered. The absorbance was recorded at 250 nm by using UV detector in HPLC (14, 18 and 31).

Table 7. Comparative Pharmacokinetic parameters of Free Drug solution & Nanoparticle Formulation

S.No	Parameters	Free drug solution	Formulation
1	Cmax	40.9 μg/ml	24.09 μg/ml
2	Tmax	6 h	12 h
3	AUC	375.44μg. h/ml	450.029 μg. h./ml
4	AUMC	3521.09μg. h^2/ml	4424.04 μg. h^2/ml
5	MRT	9.3 h	16.6822 h
6	Elimination rate constant	0.2197 h^{-1}	0.091 h^{-1}
7	Elimination t	3.154 h^{-1}	8.09 h^{-1}
8	Absorption rate constant	0.2567 h^{-1}	0.091 h^{-1}
9	Absorption $t_{1/2}$	2.699 h^{-1}	7.579 h^{-1}

3.15Pharmacokinetic data analysis
Plasma concentration-time profiles were evaluated by extra vascular method of residuals. The following pharmacokinetic parameters were determined: Cmax and Tmax (time to reach Cmax) were obtained from Kinetica 5.0 software. In case of free drug 40.9 mcg/ml drug of maximum dose was recovered but in case of nanoparticles the dose recovered in serum was 16.02 mcg / ml at the end of 6th h. The result shows that there was drastic reduction in

523

_____

the total percent of dose administered present in the serum as free drug Valsartan upon nanoparticle encapsulation; results are shown in table 7.

3.16 Stability studies

The results of stability studies of Eudragit nanoparticles of the drug VAL were shown in the table 4. The drug content of the three formulations were analysed from the initial, 2nd, 4th and 6th months. The drug content lost was about 8-15 % was observed in nanoparticles which are stored at $40^{o} \pm 2$ ^{o}C and 70 % RH, while in the formulation which was stored in $25^{o} \pm 2$ ^{o}C it was 7-13 % and in the formulation which was stored in the refrigerator condition it was found to be 1 %. Hence the formulation stored at $4^{o} \pm 1$ ^{o}C was more stable compared to the other temperatures. The results are shown in figure 10.

Figure 6. % Drug Content when drug is stored at $4^{°} \pm 2$ $^{°}$C
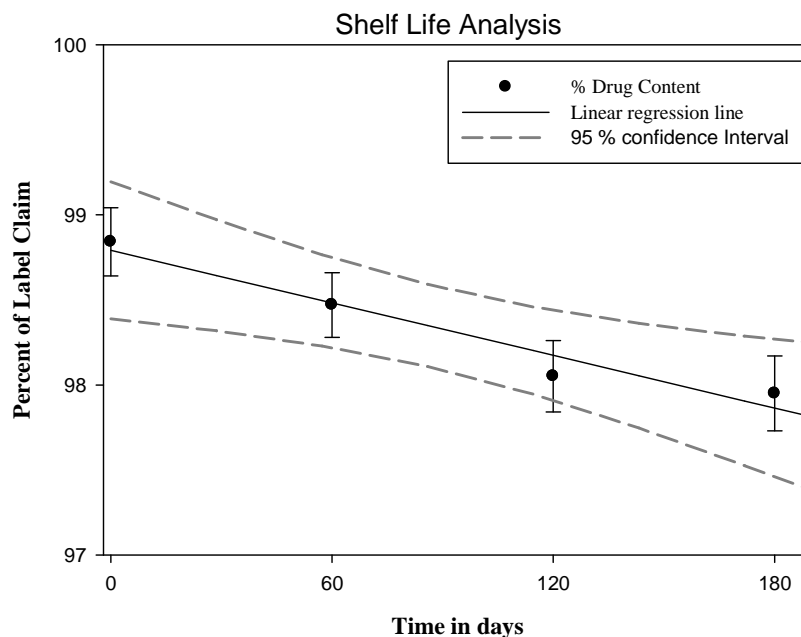
3.17 Release kinetics

The order of release of drug was found to be Zero order, in which regression value was close to 1 than value of first order equation. The Higuchi model of optimized formulation showed linear regression and it can be found that release follows diffusion kinetics mechanism. The 'n' value of Peppa`s equation was found to be less than 0.5, from this it was concluded that the drug release follows Fickian diffusion.

Data obtained from *in vitro* release studies of Eudragit nanoparticles coated with polysorbate 80 were fitted to various kinetic equations and the results are presented in table 9. The release of drug from Eudragit nanoparticles was mixed order and diffusion controlled as indicated by the higher R^2 values in the Higuchi model. Since, the 'n' values obtained from the Korsmeyer Peppa`s model were < 0.5, so the mechanism of drug release from the Eudragit nanoparticles follows Fickian mechanism. The release profile of Valsartan nanoparticles has showed slow sustained released following mixed order kinetics, and diffusion controlled with Fickian mechanism and the results are shown in table 8.

Table 8. Release kinetics

S.No	Formulation code	Zero order reaction R^2	First Order R^2	Higuchi R^2	Korsmeyer– Peppa's R^2
1	F1	0.9752	0.9817	0.9793	0.7453
2	F2	0.9877	0.9877	0.9724	0.7357
3	F3	0.9795	0.9829	0.9803	0.7521

CONCLUSION

The nanotechnology based systems may improve drug therapy of patients as demonstrated by *in vitro* and animal *in vivo* studies. Various nano systems have been shown the ability to improve bioavailability of antihypertensive activity of several drugs, while reducing their toxicity and potentially simplifying drug regimens. Also, these

systems can provide higher and prolonged drug levels in known reservoir sites. The above objectives were achieved by formulating Valsartan loaded Eudragit nanoparticles by nanoprecipitation method used for the treatment of hypertension by enhancing the bioavailability. Thus the formulation F2 has showed the sustained release formulation for the treatment of hypertension by decreasing the dose and frequency of administration and thereby reducing the side effects and improving the patient compliance.

REFERENCES

[1] Hartman RP, Kawashima A, *Am Fam Physician*, 80, 3, **2009,** 273-9.

[2] Remuzzi A, Perico N, Remuzzi G, *Drugs Today*, 34, 11, **1998**, 973-86.

[3] Cyong JC, Uebara K, *Single administration study*, 14, **1998**, 1703-1725.

[4] Flesch G, Muller P, Lloyd P, *Eur J Clin Pharmacol,* 52, 2, **1997**, 115-120.

[5] Colussi DM, Parisot C, Rossolino ML, Brunner LA, Lefèvre GY, *J Clin Pharmacol*, 37, **1997**, 214-221.

[6] Israili ZH, *J Hum Hypertens*,14, **2000,** S73- S86.

[7] Arnaud C, Michel B, Expert opinion on investigational drugs, 7, 11, **1998**, 1915-1925.

[8] Jensen CE, Dos Santos RA, Denadai AM, Santos CF, Braga AN, Sinisterra RD, *Molecules*, 4, 15, 6, **2010**, 4067-84.

[9] Venkatesh kumar, Arun Kumar N, Verma PRP, Rani C, *Int J PharmTech Res*, 3, **2009**, 431-437.

[10] Selvakumar K, Yadav AV, *Int J PharmTech Res*, 2, **2009**, 179-183.

[11] Gowda DV, Srikrishna RM, Shivakumar HG, *Indian Journal of Pharm Edu Research*, **2009**, 43, 1.

[12] Trapani A, Denora N, Iacobellis G, Sitterberg J, Bakowsky U, Kissel T, *AAPS pharmscitech*, **2011**.

[13] Tamizhrasi S, Shukla A, Shivakumar T, Rathi V, Rathi JC, *Int journal of pharmtech research*, 1, **2009**, 411-415.

[14] Imanishi T, Tsujioka H, Ikejima H, Kuroi A, Takarada S, Kitabata H, Tanimoto T, Muragaki Y, Mochizuki S, Goto M, Yoshida K, Akasaka T, *Hypertension,* 52, 3, **2008**, 63-72.

[15] Padma VD, GaneshChandra SS, *Drug Dev Ind Pharm*, 33, **2007**,101-111.

[16] Pereira R, Julianto T, Kah Hay Yuen Majeed ABA, International Conference on Nanoscience and Nanotechnology. IEEE Xplore. (**2006**) (doi:10.1109/ICONN.2006.340520).

[17] Pignatello R, Ricupero N, Bucolo C, Maugeri F, Adriana M, Puglisi G, *AAPS Pharm SciTech,* 7, **2006**, E1-E7.

[18] Macek J, Klíma, Tacek P, *J Chromatogr B AnalytTechnol Biomed Life Sci*, 832, 1, **2006**, 169-72.

[19] Hannele E, Leena P, Janne R. Jouni Hirvonen EI, Kauppinen, *AAPS PharmSciTech,* 68, **2004**, 1-9.

[20] Abadir PM, Carey RM, Siragy HM, *Hypertension*, 42, **2000**, 4, 600-4.

[21] Kumar SS, Rukmani K, *Acta pharmaceutica Turcica*, 45, **2003**, 125-130.

[22] Peltonen L, Koistinen P, Karjalainen M, Häkkinen A, Hirvonen J, *AAPS Pharm SciTech*, 3, 4, **2002**, E1-E7.

[23] Barbault S, Gref R, Russo P, Guechot J, Bochot A, *J Control Rel,* 83, 3, **2002,** 365-375.

[24] Cavalli R, Caputo O, Carlotti E, Trotta M, Scarnecchia C, Gasco MR, *Int J Pharm*, 148, **1997**, 47-54.

[25] Jores K, Mehnert W, Drechsler M, Bunjes H, Johann C, Maeder K, *J Controlled Release,* 95, **2004,** 217-227.

[26] Peltonen L, Koistinen P, Hirvonen J, *S T P Pharma Sci*, 13, 5, **2000**, 299-304.

[27] Pecora R, *J Nanoparticle Res*, 2, 2, **2003,** 123-131.

[28] Waterman KC, *Pharm Dev Technol*. 12, 1, **2007,** 1-10.

[29] Suryakant B, Jadhav, Dinesh M, Sakarkar, Datta R, Kaudewar, *Der Pharmacia Sinica*, **2011**, 2 ,6,23-31.

[30] Anusha V, Palanichamy S, Sugumar M, Rajesh M, Parasakthi N, Godwin Raja Das T, Ramasubramaniyan P, Thanga Thirupathi A, *Der Pharmacia Sinica*, 3, 2, **2012**, 211-216.

[31] Costa P, Lobo JMS, *Eur J Pharm Sci* 13, 2, **2001**, 123-133.

[32] Dipak K, Chowdhury Ashim, Mitra DK, *Int J Pharm,* 193, 1, **1999,** 113-122.

[33] Nithiyananthan TS, Shankar ananth V, Rajasekhar KK, Ravikiran P, Vikram kumar E, Jayanth kumar RG, *DIT*. 1, 2, **2009**, 154-156.