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Self-nanoemulsifying Drug Delivery Systems of Valsartan: Preparation and *In-Vitro* Characterization

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Abstract

The main objective this study is to prepare and evaluate the selfnanoemulsifying drug delivery (SNEDDS) system in order to achieve a better dissolution rate of a poorly water soluble drug valsartan. The present research work describes a SNEDDS of valsartan using labrasol, Tween 20 and Polyethylene glycol (PEG) 400. The pseudo-ternary phase diagrams with presence and absence of drug were plotted to check for the emulsification range and also to evaluate the effect of valsartan on the emulsification behavior of the phases. The mixtures consisting of oil (labrasol) with surfactant (tween20), co-surfactant (PEG 400) were found to be optimum formulations. Prepared formulations were evaluated for its particle size distribution, nanoemulsifying properties, robustness to dilution, self emulsication time, turbidity measurement, drug content and *in-vitro* dissolution. The optimized formulations are further evaluated for heating cooling cycle, centrifugation studies, freeze thaw cycling, particle size distribution and zeta potential were carried out to confirm the stability of the formed SNEDDS formulations. The prepared formulation has a significant improvement in terms of the drug solubility as compared with marketed tablet and pure drug, thus, this greater dissolution of valsartan from formulations could lead to higher absorption and higher oral bioavailability.

Keywords: Self Emulsifying Drug Delivery System, Nanoemulsion, Valsartan, Enhancement of dissolution, poorly soluble drug,

Introduction

Over the decades, oral drug delivery system had been a very favourable and most explored field in pharmaceutical technology due to its convenience in administration. However, one of the limitations is that different drugs exhibit distinct and different delivery profile from each another. One of the most important factors for such phenomenon is the drugs' solubility.

One of the drugs struggling with solubility is Valsartan. Valsartan (N-pentanoyl-N-{[2'-(1H-tetrazol-5-yl) biphenyl-4-yl] methyl}-L-valine) is an angiotensin II receptor blocker [1]. It is indicated for hypertension, heart failure and post-myocardial infarction [2]. According to United States Pharmacopeia, Valsartan in capsule form has the bioavailability of approximately 25% (range 10 – 35%). This phenomena might be attributed to the low water solubility of valsartan (log P = 1.449) [3]. However, Valsartan is classified under Biopharmaceutical Classification System class III, which is a drug with low permeability, poor metabolism and high solubility [4]. This is because Valsartan is not entirely lipophilic and it exhibits pH-dependent solubility, where the solubility is 16.8 g/L and 0.18 g/L in phosphate buffer solution (PBS) pH 8.0 and water respectively. Being a weakly acidic drug (pKa = 8.15), Valsartan is generally in the ionized form at higher pH, and thus greater solubility. [4], [5] It should be noted that drugs can only cross the intestinal lining at its unionized and solubilised form. At the lower pH in the stomach, Valsartan will mainly exist in the unionized form which can facilitate permeation but drug solubility is the limiting factor. [3], [4] Therefore, it is plausible that by addressing the solubility issue at low pH, enhanced absorption of Valsartan and thus greater bioavailability can be achieved.

Self-emulsifying drug delivery system is one of the effective systems in addressing solubility issues. Self-nanoemulsifying drug delivery system (SNEDDS) is composed of an isotropic mixture of oil, surfactant, co-surfactant and drug [6-7]. Upon ingestion, this isotropic mixture will come in contact with the aqueous phase of gastrointestinal tracts and form an oil-in-water emulsion at a nano-scale range with the aid of gastrointestinal motility. This stable emulsion can provide a large interfacial area for partitioning of drug between oil and aqueous phase and potentially offer better dissolution rate and improved bioavailability [8].

SNEDDS appears to be an attractive choice of formulation as it requires simple and cost-effective manufacturing facilities. [8] This is because SNEDDS is a physically stable lipid solution and it omits the need of high energy emulsification process, and thus reduces the manufacturing cost. In addition, better dissolution rate and

more predictable bioavailability of SNEDDS imply the reduction in drug dose and possibly eliminate the dose-related side effects [9-10]. Many of the past researches had been done on this attractive choice of delivery, mostly on chemical entities which are poorly water soluble, such as all-trans-retinol acetate [11], carvedilol [12], flutamide [13,14] and fenofibrate [15] showed more than 90% of cumulative drug release. Based on the above fact the study was aimed to prepare and characterize SNEDDS of poorly soluble drug valsartan using optimized choice and ratio of oil, surfactant and co-surfactant to improve the rate of dissolution drug which leads to increase of bioavailability of drug.

Material and Methods

Materials

Valsartan is purchased from Aurobindo Pharma Ltd, India. Capryol 90, Labrafil M 1944 CS, Labrafac Lipophile WL 1349(504), Labrasol and Transcutol HP are gift samples from Gattefosse ®, France. Tween 20, Tween 40 and Tween 60 are purchased from R&M Chemical, UK. PEG 300, PEG 400 and PEG 600 are manufactured by Aldrich ®, Germany, while is purchased from Sigma-Aldrich chemicals, USA. Olive oil purchased from Fluka ® chemicals, USA. Other analytical solvent used are of analytical grade. The marketed product is purchased with legal prescription and the name is not revealed due to confidentiality.

Solubility Studies

The first step of this experiment design is to screen the solubility of poorly soluble Valsartan in each of the individual oils, surfactants and co-surfactants. The solubility of Valsartan in various oils is determined by adding excess amount of drug in 2 mL of selected oils, surfactants and co-surfactants into 5mL vials, and mixed thoroughly via vortex mixer [16]. The vials are then kept at 25 °C in a shaker for 24 hours. After that, the mixtures were spun using a centrifugator (Centrifuge Bench Top Refrigerated, Eppendorf) at 3000 rpm for 15 minutes. The supernatants were retrieved and quantified using UV-Vis spectrophotometer at 251nm [17-18] (Lambda 25, Perkin Elmer).

Pseudo-ternary Phase Diagram

Based on the individual solubility studies and the hydrophilic-lipophilic balance (HLB) value, the oil, surfactant and co-surfactant are chosen for the construction of the pseudo-ternary phase diagram [16]. The surfactant and co-surfactant are mixed in different ratio (1:1, 2:1, and 3:1). Phase diagrams are constructed for each ratio. For every phase diagram, the proportion of oil and Smix varies in different volume ratio from 0:10 to 10:0. The percentage of the aqueous phase is determined using aqueous titration method [19]. Slow titration of distilled water is added (5% addition at a time) into the Smix-oil mixture and the observation of the transition from clear to turbid point is noted down. Calculation is made to determine the percentage of water, oil and Smix present at the point of turbidity. With the obtained individual percentage, a pseudo-ternary phase diagram is developed with the clear-solution region marked as best emulsification region. The six formulations are selected from different region of emulsification area.

Preparation of Self-nanoemulsifying Drug Delivery System

Aqueous titration was repeated for the selected Smix ratio in the presence of valsartan. Another pseudoternary phase diagram was constructed in presence of drug as show in Fig.3 and six points were randomly picked from the self-nanoemulsifying region. The percentage of each component for all six points was calculated and presented in Table 1. Correspondingly, six formulations were prepared by mixing all specifically measured oil, surfactant, co-surfactant and 80mg of valsartan.

Thermodynamic stability testing

The formulations were subjected to heating-cooling, centrifugation and freeze-thaw, where the physical appearances of the formulations were observed at the end of each testing. In heating cooling, all six formulations were heated at 45°C and then cooled at 4°C, with the duration of 24 hours at each temperature, for 2 cycles. Then, formulations which passed the heating-cooling cycles were subjected to centrifugation at 3500 rpm for 15 minutes. Finally, only formulations which passed the previous two steps were stored at alternating temperature of -21°C and 25°C, with the duration of 24 hours at each temperature, for 2 cycles.

Robustness to dilution

The prepared formulations were diluted infinitely (i.e. 900 times) with 4500 µl of water, Phosphate buffer pH 6.80 and acid buffer pH 1.2 in three separate glass vials. The diluted formulations were shaken and then visually inspected after 24 hours for any form of instability [16].

Droplet size and zeta potential analyses

This analysis was carried out so as to determine the consistency in the size and stability of the emulsion at various dilutions (i.e. 100, 500 and 900 dilution factors) and dispersant media (miliQ water, pH 6.80 PBS and pH 1.20 acid buffer). Malvern Zeta Sizer Nano ZS with the conditions of backscatter detection at 173º; temperature of 25ºC; refractive index of 1.330 were used. All were done in triplicates.

Transmission Electron Microscopy (TEM)

A drop of diluted formulation was placed on a carbon-coated copper grid, stained with 2% uranyl acetate aqueous solution, and examined using the TEM (Philips Tecnai 12).

In vitro dissolution studies

Dissolution studies were carried out using USP Apparatus Type II (paddle type) with 900 ml of pH 6.80 ± 0.05 PBS, temperature at 37 ± 0.5ºC and paddle rotation of 50 rpm. 5 ml of formulation, which contained 80 mg of valsartan, was instilled to the dissolution medium at time 0 minute. 5 ml of dissolution media was retrieved at timed intervals and the amount of valsartan was quantified using HPLC method.

The HPLC analysis is performed using Shimadzu HPLC model LC-10ADvp, equipped with Shimadzu degasser model DGU-14A; system controller SCL-10Avp; fluorescence detector RF-10A XL; auto injector SIL-10AD vp; column oven CTO-10AS and Prominence Diode Array (PDA) detector SPD-M20A. The chromatographic column used was Symmetry Shield RP18 C18 (250mm ˣ 4.6mm, 5µm particle size) and the mobile phase was prepared with ammonium dihydrogen phosphate buffer: methanol (2:1) adjusted to pH 3.0 with formic acid. [17] The solvents used are of HPLC grade. Dissolution studies were also done using acid buffer pH 1. All were done in triplicates.

Emulsification time

Under the same conditions as in vitro dissolution studies, time taken by the formulation to form homogenous mixture with the dissolution medium was noted in triplicates. The assessment for the efficiency of the emulsion system is also made according to the following grading system

Grade A: Rapidly forming emulsion having a clear or bluish appearance

Grade B: Rapidly forming, translucent bluish appearance

Grade C: Fine milky emulsion forming within 2 minutes

Grade D: slow forming, slightly oily appearance

Dispersibility testing

Under the same conditions as in vitro dissolution studies, the type of emulsion formed was visually inspected and categorised as either clear, translucent with bluish tone or milky turbid emulsion.

Accelerated stability testing

All anhydrous formulations were stored in an incubator at 40ºC and 75% relative humidity for four weeks. Visual assessment, droplet size and zeta potential analyses were conducted for selected formulations at the end of the study.

Result and Discussion

Solubility studies

Based on the solubility profile of different oil, surfactant and co-surfactant and pre-formulation studies about the interaction between drug-excipients as seen in Fig.1., we have selected labrasol as oil with 108.9 mg/ml of solubility, Tween 20 as surfactant with 47.7mg/ml solubility and PEG 400 as co-surfactant for preparation the SNEDDS formulations.

As for the selection of Smix, these components must be of GRAS status, which suggested safe for oral consumption. The combination of oil, surfactant and co-surfactant were ensure to be able to solubilise the required amount of drug (80mg at least). Besides, another determining factor for the formulation was the hydrophilic-lipophilic balance (HLB) value [20]. According to William C. Griffin's method,the desired HLB value to form an oil-in-water emulsion should be between 8-18[21]. Also, there were concerns regarding the solubility of Valsartan in the mixture in highly hydrophilic mixture. Upon calculation of selected smix were found to have HLB values of approximately 14 which is suitable for the preparation oil-in- water type of nanoemulsion that can contain 80mg of valsartan.

Construction of pseudo-ternary phase diagram

On the basis of the solubility of drug, oil, surfactants, co-surfactants and aqueous phase were used for construction of phase diagram. Oil, surfactant, and co-surfactant are grouped in four different combinations for phase studies. Aqueous titration was used for the construction of pseudo-ternary phase diagram. The titration was done in a way that each aqueous addition with water was approximately 5% increment up to 95% [19]. As shown in Fig .2, the self-nanoemulsifying regions appeared to increase with decreases marginally as increases Smix ratio.

The Smix 1:1 resulted in the largest self-nanoemulsifying region than that of 3:1 as shown in Fig.3. It was also noticeable that 1:1 ratio of Smix, the mixture can take up greater amount of water and still remain as clear mixture with bluish tone during aqueous titration. This could be explained by the fact that higher amount of surfactants can be adsorbed at the interface and hence, stabilized the formation of nanoemulsions. Nanoemulsions appeared transparent as their droplet radii fell below the optical wavelength of visible light (390 - 750 nm) by which minimum light scattering took place and the bluish tone was due to the dominance of low-wavelength light scattered from them [22]. As the droplet radius approached 100 nm, nanoemulsions seemed hazy, and above this, in the submicron range, they appeared white due to significant multiple light scattering

The transparency of the emulsion was believed to be inversely proportional to the size of the droplets, i.e. the more transparent the emulsion is, the smaller the size of the droplets. [23] This is because the droplet radii were believed to fall below the optical wavelength of visible light with minimal light scattering. However, no droplet size analysis was done to confirm the statement above. Also, at certain aqueous percentage, appearance of the mixture changes for smooth flowing to viscous gel-like appearance, which was undefined as an emulsion.

Formulations of SNEDDS

No significant ($p<0.05$) different in self-nanoemulsifying region in presence of 80 mg of valsartan and in absence of valsartan was

observed as shown in Fig.3. Six points were randomly picked from the self-nanoemulsifying region and the formulations were summarized in Table 1

Droplet size analysis

The droplet size was the main target of the entire formulation of study, where it was postulated that the smaller the droplet size, the larger the interfacial area, thus the greater the partitioning of the drug across the gastrointestinal lining. The droplet size of all the formulations were examined at different dilutions, i.e. 100, 500 and 900 times dilution with water and the results are presented in Table 2. The purpose was clear, which is to detect any changes in droplet size with the increasing dilutions. However, there was no consensus on the exact size range of nanoemulsion. In the present study, average droplet size of formulation F1 and F2 were found to be less than 200 nm with low polydispersity index (<0.5) as shown Table 2. The result would show a clear picture regarding \stability behaviour of the emulsion within the gastrointestinal tract, where the consistency in the emulsion droplet size was observed.

Effect of droplet size and zeta potential in different dispersant media

Due to considerable pH variations along gastrointestinal tracts, it is rational to observe the consequence of different media on the SNEDDS. Although the droplet sizes of the formulations were found to be less than 200nm with low polydispersity index (<5) as shown in Table 3.

The droplet size of formulations changes marginally at different dispersant media (Table 3). The droplet size is increases when it disperses in acid buffer pH1.2 as compared to water PBS pH6.8 as seen in Table 3. This may be due to the weakly acidic valsartan, which was mainly unionised at lower pH, which remained in the oil droplets and hence resulting in bigger droplet size at acidic condition. As opposed, the weakly acidic valsartan was mainly ionised at higher pH, had the tendency to diffuse to the continuous phase of PBS, resulting in smaller droplet size at basic condition [23]. The physical stability of the emulsions can be projected through the zeta potential analysis. Zeta potential is an indication of the degree of repulsion between the individual droplets in the entire emulsion. The zeta potential of formulation was found to be in the range between +3.98 to -19.45 which would be better the stability of the emulsion as the individual droplets repels each another to coalescence into larger globules [24-25].

Transmission Electron Microscopy (TEM)

Microscopy analysis revealed well-defined circular globules with the size of less than 100 nm as shown in figure 4. The TEM photo graph indicated that the formed O/W nanoemulsion globules has smooth surface with no surface drug crystals.

Robustness to dilution/precipitation

The dilution capability of the formulations was tested to determine the capability of the formulation to withstand possibly infinite dilutions. This was because upon ingestion, the gastrointestinal fluids are responsible for the dilution, and it is impossible to accurately identify the amount of water present to form emulsion with the formulation Robustness to dilution was performed diluted with excess of water, standard phosphate buffer pH 6.8 and 0.1N HCl (500-900 ml) and was stored for 12 hours and result were indicated that there was no precipitation or phase separation as shown in Table 4.The ability of SNEDDS formulation to withstand aqueous dilution was found to be fascinating. The phenomenon was attributed to the high solubilising properties of the excipients, and also the capability to form a relatively stable emulsion with small droplet sizes [24]. This implied that these formulations were stable at infinite water dilution.

In- vitro dissolution studies

The *in-vitro* dissolution studies of formulations (F1 & F2), Diavon tablet and Valsartn drug powder were performed in acidic buffer pH 1.2. The dissolution profiles of formulations were compared with marketed tablet (Diovan tablet) and valsartan drug powder and results are shown in fig 5 and 6.

The maximum drug release from formulation F1 and F2 were found to be 86.12 % and 86.46% , respectively at end of 40 min, whereas the maximum drug release from marketed valsartan tablet and valsartan powder were found to be 14.36% and 7.45%, respectively at end of 40 min. The results from figure 5 and 6 is clearly indicates that rate and extent of dissolution of prepared formulations (F1 and F2) were significantly ($p<0.05$) higher than that of marketed valsartan tablet and valsartan powder.

It could be suggested that the SNEDDS formulation resulted in spontaneous formation of a nanoemulsion with a small droplet size, which permitted a faster rate of drug release into the aqueous phase, significantly much faster than that of marketed valsartan tablet and powder.

Thermodynamic stability testing

The thermodynamic results of the formulation in shown in Table 5. All of SNEDDS formulations were passed the thermodynamic stability testing as there was no sign of phase separation or drug precipitation at the end of all cycles

. This suggested that the formulations were robust against storage at extreme conditions.

Emulsification time

The most of the prepared SNEDDS formulations were formed the nanoemulsion in less than 1 min with grade A and B as shown in Table 6. The formulation F1 and F2 were formed the nanoemulsion within 30 seconds with grade A system in PBS and

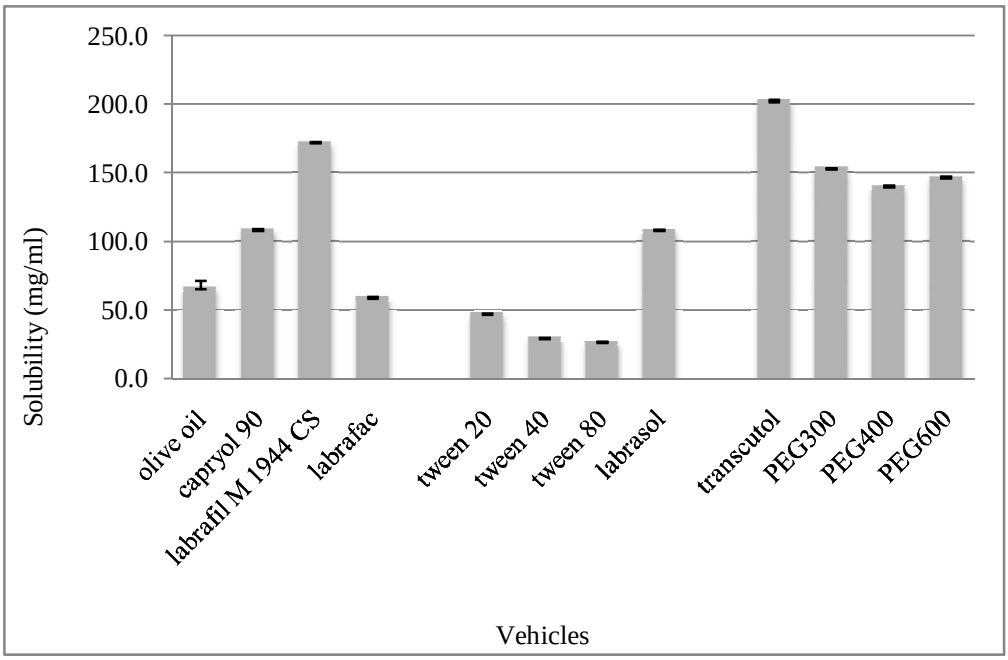

Fig.1. Saturation solubility valsartan in different oil, surfactants and Co-surfactants

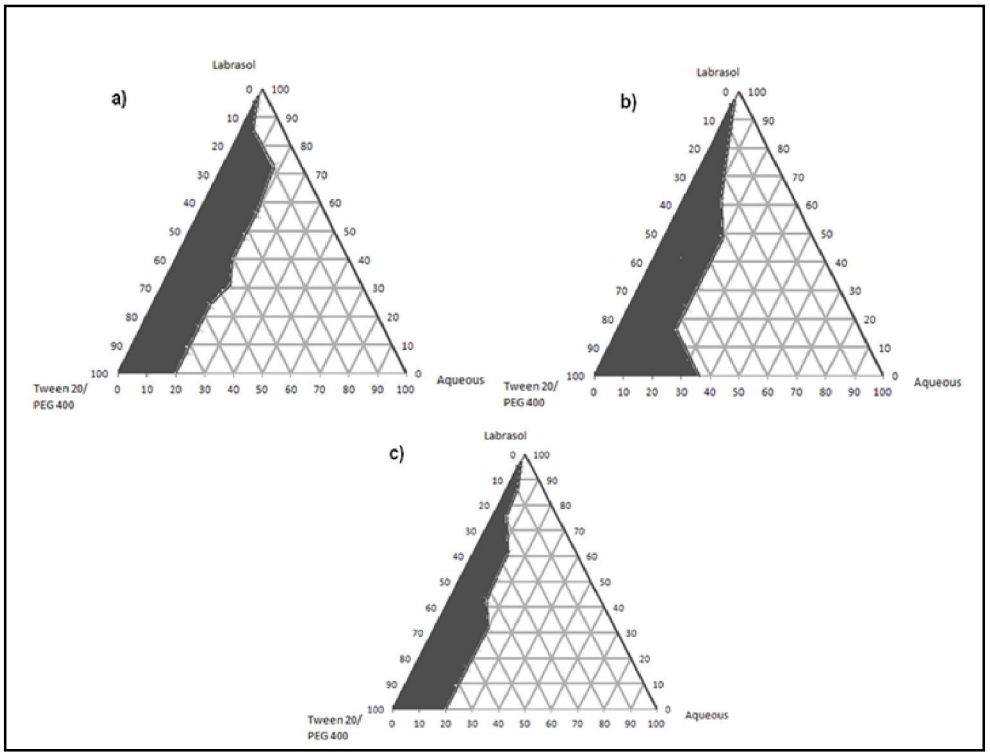

Fig.2. Pseudoternary phase diagrams of Labrasol, Tween 20 and PEG 400 (Smix) and water, in the absence of valsartan, with Smix ratios: a) Smix 1:1; b) Smix 2:1 and c) Smix 3:1.

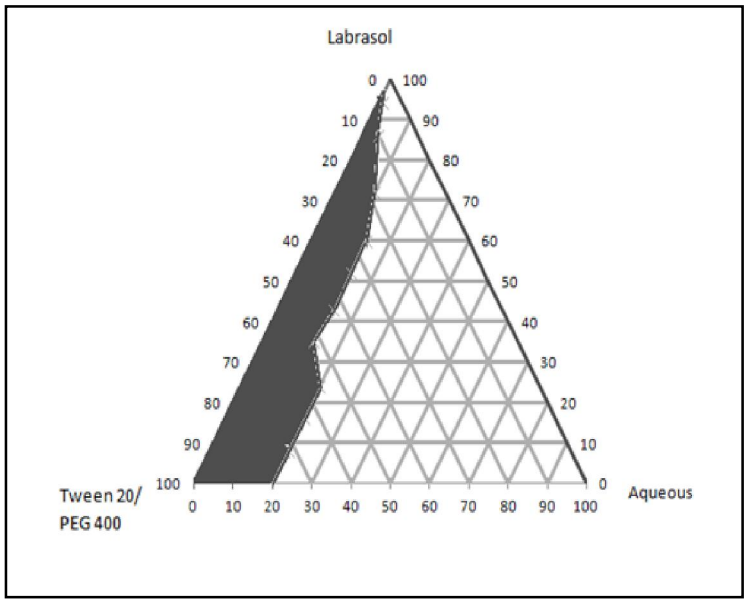

Fig.3. Pseudoternary phase diagrams of Labrasol, Tween 20 and PEG 400 (Smix) and water in the presence of 80mg of valsartan with 1:1 smix ratio.

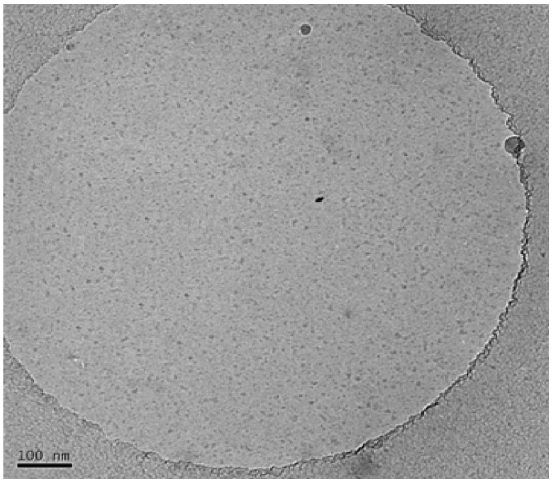

Fig.4. TEM image of formulation E upon 100 time dilutions with water.

powder and results are shown in fig 5 and 6

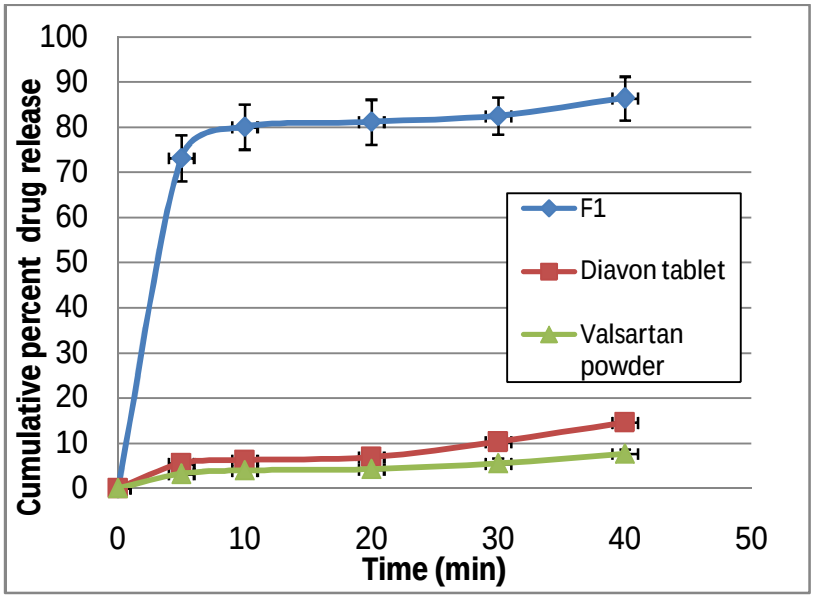

Fig.5. *In vitro* dissolution profile of formulation F1 as compared with Diavon tablet and valsartan powder using acid buffer pH 1.2.

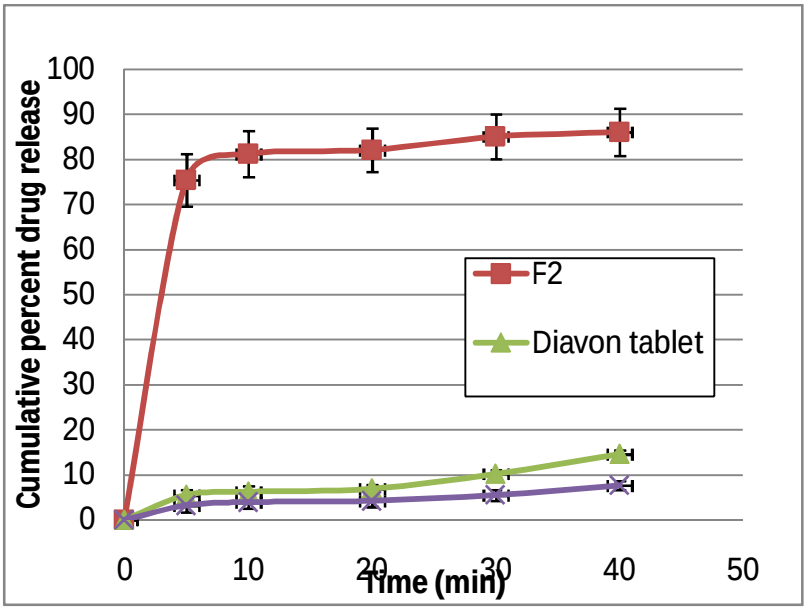

Fig.6. *In vitro* dissolution profile of formulation F2 as compared with Diavon tablet and valsartan powder using acid buffer pH 1.2.

Table.1. Percentage of each component in the selected six formulations.

Formulation	Labrasol (% v/v)	Tween 20 (% v/v)	PEG 400HP (% v/v)
F1	50.5	25.0	25.0
F2	67.0	16.5	16.5
F3	50.0	37.5	15.5
F4	40.5	48.5	16.0
F5	25.0	25.0	19.0
F6	45.5	42.5	20.5

Table.2. The average droplet size of all six formulations at various dilution factors with water (n=3).

Formulation	Dilution factor	Droplet size (nm)	PDI
F1	100	94.41 ± 1.45	0.421 ± 0.024
F1	500	89.47 ± 0.98	0.345 ± 0.016
F1	900	84.15 ± 1.14	0.368 ± 0.031
F2	100	114.47 ± 1.45	0.347 ± 0.005
F2	500	106.45 ± 2.45	0.381 ± 0.089
F2	900	104.75 ± 2.47	0.346 ± 0.085

Table.3. The average droplet size, PDI and zeta potential of selected formulations at 900 times dilution with various dispersant media (n=3).

Formulation	Dispersant media	Droplet size (nm)	PDI	Zeta potential (mV)
F1	Water	94.41 ± 1.65	0.345± 0.044	-18.45 ± 0.46
F1	Acid buffer pH 1.2	117.14 ± 1.45	0.341 ± 0.033	-10.45 ± 0.21
F1	PBS pH 6.8	114.63 ± 2.45	0.421 ± 0.014	15.17 ± 0.47
F2	Water	108.47 ± 1.45	0.347 ± 0.005	-16.90 ± 1.57
F2	Acid buffer pH 1.2	122.85 ± 2.24	0.526 ± 0.017	-12.80 ± 1.11
F2	PBS pH 6.8	101.21 ± 1.41	0.557 ± 0.045	3.98 ± 0.71

Table.4. Robustness to dilution of formulation in different dissolution medium.

Dissolution medium	F1	F2	F3	F4	F5	F6
Water	P	P	P	P	P	P
Acid buffer pH 1.2	P	P	P	P	F	P
Phosphate buffer pH 6.8	P	P	P	P	P	P

P = Passed, F= Failed

Table.5. Thermodynamic stability studies of SNEDDS formulation under different stability conditions

Formulations	Heating cooling cycle	Centrifugation	Freeze thaw cycle
F1	P	P	P
F2	P	P	P
F3	P	P	P
F4	P	P	F
F5	F	P	P
F6	P	P	P

P = Passed, F= Failed

Table.6. Assessment of self- emulsification parameters of formulation in dissolution medium.

Formulations	Self-emulsification time (Seconds)	Assessment of self-emulsification
F1	18.45 ± 1.74	Grade A
F2	13.45 ± 1.34	Grade A
F3	28.12 ± 1.44	Grade B
F4	36.45 ± 1.82	Grade D
F5	42.45 ± 1.46	Grade D
F6	48.78 ± 1.44	Grade C

Table.7. The droplet size, PDI and zeta potential of formulation F1 and F2 before and after accelerated stability study (n=3).

	Before accelerated stability study			After accelerated stability study		
Batch	Droplet size (nm)	PDI	Zeta potential (mV)	Droplet size (nm)	PDI	Zeta potential (mV)
F1	94.41 ± 1.65	0.345± 0.044	-18.45 ± 0.46	98.42± 1.28	0.328 ± 0.12	-19.45 ± 0.28
F2	108.47 ± 1.45	0.347 ± 0.005	-16.90 ± 1.57	119.10± .94	0.347 ± 0.02	-16.44 ± 1.87

acid buffer. The results are indicating that the prepared SNEDDS formulations form a good and stable nanoemulsion in different dissolution with short time duration (<1 min).

The accelerate stability test was performed at 45°C/75RH for 8weeks. There was no observation of phase separation, drug precipitation or colour change in formulations F1 and F2 at the end of 8-weeks accelerated stability conditions. Particle size and Zeta potential measurement also revealed similar droplet size ($p>0.05$) and relatively stable droplets as shown in Table 7.

Conclusion

The SNEDDS containing valsartan was successfully prepared using Labrasol (oil) Tween 20 (surfactant) and PEG 400 (co-surfactant). The prepared formulations were formed nanoemulsion in different pH conditions with particle size~100nm. Furthermore, the results are indicated that the prepared formulations are having a good stability in the terms of droplet size, zeta potential under different dispersant media and stability conditions. This formulation showed significant improvement in rate of dissolution of valsartan in acidic buffer pH1.20 which is more than 6-fold of drug release as compared to marketed valsartan tablet. Thus, this greater dissolution of valsartan from the SNEDDS formulation could lead to higher absorption and higher oral bioavailability.

Conflict of Interest

There is no conflict of interest

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References

[1]. Zaid AN et al. Formulation and bioequivalence of two Valsartan tablets after a single dose administration. Scientia Pharmaceutica 2011; 79: 123 – 135.

[2]. Novitch M. The United States Pharmacopoeia: The National Formulary Asian Edition. Tata Press Limited. USA: 1994.

[3]. Siddiqui N et al. Pharmacacological and pharmaceutical profile of Valsartan: A Review. Journal of Appl Pharmal Sci. 2011; 01 (04): 12 – 19.

[4]. Saydam M, Takka S. Bioavailability file: Valsartan. FABAD J Pharm Sci 2007; 32: 185 – 196.

[5]. DrugBank: Open Data Drug & Drug Target. 2007. Available from: http://www.drugbank.ca/drugs/DB00177

[6]. Patel SN et al. Self-emulsifying drug delivery system. J Global Tech 2010; 2(2): 29 – 37.

[7]. Tang J, Sun J, He ZG. Self-Emulsifying Drug Delivery Systems: Strategy for improving oral delivery of poorly soluble drugs. Current Drug Therapy 2007; 2: 85 – 93.

[8]. Date AA, Desai N, Dixit R & Nagarsenker M. Self-nanoemulsifying Drug Delivery Systems: Formulation insights, applications and advances. Nanomedicine 2010; 5(10).

[9]. Nielsen FS, Petersen KB & Müllertz A. Bioavailability of Probucol from lipid and surfactant based formulations in minipigs: influence of droplet size and dietary state. Eur J Pharm Biopharm 2008; 69: 553-62.

[10]. Kumar GP, Rambhau D, Aapte SS. Oral bioavailability enhancement of Carbamazepine in healthy human volunteers. J Pharm Research 2011 Jan 13rd; 4(2): 361-5.

[11]. Taha EI, Al-Saidan S, Samy AM, Khan MA. Preparation and in vitro characterization of self-nanoemulsified drug delivery system (SNEDDS) of all-trans-retinol acetate. Intr J Pharm 2004; 11(285)109-19.

[12]. Mahmood EA, Bendas ER, Mohamed MI. Preparation and evaluation of self-nanoemulsifying tablets of Carvedilol. AAPS 2009 Feb 24; 10(1):9192-7.

[13]. Jeevana JB & Sreelakshmi K. Design and evaluation of self-nanoemulsifying drug delivery system of Flutamide. J Young Pharm 2011; 3(1): 4-8.

[14]. Dixit AR, Rajput SJ & Patel SG. Preparation and bioavailability assessment of SMEDDS containing Valsartan. AAPS 2010 Feb; 11(1):314-21

[15]. Patel AR, Vavia PR. Preparation and in vivo evaluation of SMEDDS (Self-Microemulsifying Drug Delivery System) containing Fenofibrate. AAPS Journal 2007; 9(3): 344 – 352.

[16]. Gupta AK et al. Preparation and in-vitro evaluation of self-emulsifying drug delivery system of antihypertensive drug Valsartan. Intr J Pharma and Life Sciences March 2011; 2(3): 633 – 639.

[17]. Parambi DGT, Mathew M, Ganesan V. A validated stability indicating HPLC method for the determination of Valsartan in tablet dosage forms. Journal of Applied Pharmaceutical Science. June 2011; 01(04): 97 – 99.

[18]. Dey S et al. Development and validation of a UV-Vis Spectrophotometric method for the estimation and degradation monitoring of Cefradroxil in bulk and pharmaceutical dosage forms. International Journal of Chemistry Research. May 2010; 1(1): 29 – 35.

[19]. Shafiq-un-Nabi S et al. Formulation development and optimization using nanoemulsion technique: A technical note. AAPS PharmSciTech 2007; 8(2): Article 28.

[20]. Griffin WC. Calculation of HLB values of non-ionic surfactants. J. Soc. Cosmet. Chem. (1954): 259.

[21]. Pasquali RC, Taurozzi MP & Bregni C. Some considerations about the hydrophilic-lipophilic balance system. Int. J. Pharm 2008 May 22; 356(1 – 2): 44 – 51.

[22]. Pouton CW. Formulation of self-emulsifying drug delivery systems. Adv Drug Deliv Rev 1997; 25: 47 – 58.

[23]. Particle Sciences Drug Development Services. Emulsion stability and testing. Tehnical Brief 2011 Volume 2. [cited 2011 July 30]. Available from: http://www.particlesciences.com/docs/technical_briefs/TB_2011_2.pdf

[24]. Colloidal Dynamics. The zeta potential. [cited 2011 July 27]. Available from: http://www.coloidal-dynamics.com/docs/CDElTUT1.pdf

[25]. Mason TG, Wilking JN, Meleson K, Chang CB & Graves SM. Nanoemulsions: Formation, structure and physical properties. J. Phys: Condens. Matter 18 (2006) R635–R666.