Research Article

Development of Microemulsion for Solubility Enhancement of Poorly Water Soluble Drug Valsartan

Shruti G. Shahu*, Rita N. Wadetwar, Gouri R. Dixit
J. L .Chaturvedi College of Pharmacy, Electronic Zone Building, MIDC, Hingna Road, Nagpur, India.
***Corresponding author's E-mail:** shrutishahu1@gmail.com

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ABSTRACT

Valsartan is orally active, and specific angiotensin II antagonist acting on the AT1 receptor subtype (angiotensin receptor blocker). Valsartan is poorly water soluble drug. The aim of the investigation was to design and develop micro emulsion of valsartan for enhancing its solubility hence the oral bioavailability. Solubility of valsartan was determined in various vehicles and maximum solubility was found in Capmul MCM (oil), Cremophor EL (surfactant) and Transcutol HP (co-surfactant). These components were used to construct pseudo-ternary phase diagrams to identify the micro emulsion existing zone. The micro emulsion was prepared by phase titration method. Optimized micro emulsion was characterized for its transparency, particle size, drug content, viscosity, and stability study etc. Particle size of optimized micro emulsion was found to be 51.32 nm. Drug content of the micro emulsion formulation was 98.29% ± 0.91%. The viscosity data indicated the micro emulsion to be O/W type. 78.49% and 71.53% of the drug was found to be released in 4hrs in the *in-vitro* and *in-vivo* intestinal permiability studies respectively. Hence, by formulating into micro emulsion, the solubility of valsartan was significantly enhanced which may increase its bioavailability. Thus, it could be concluded that micro emulsion formulation could be used as a possible alternative to traditional oral formulations of Valsartan to improve its bioavailability.

Keywords: Micro emulsion, Pseudo-ternary phase diagrams, Solubility, Valsartan.

INTRODUCTION

Oral route still remains the favorite route of drug administration in many diseases and till today it is the first way investigated in the development of new dosage forms. Successful oral delivery of drugs has always remained a challenge to the drug delivery field, since approximately 40% of the new drug candidates have poor water solubility, and thus oral delivery is frequently associated with implications of low bioavailability.[1]

In recent years, much attention has focused on lipid-based formulations to improve the oral bioavailability of poorly water soluble drug compounds. In fact, the most popular approaches is the incorporation of the active lipophillic component into inert lipid vehicles such as oils, surfactant dispersions, nano emulsions, micro emulsions, self-emulsifying formulations, self nano or micro emulsifying formulations, emulsions and liposomes.[2]

Valsartan is a nonpeptide, orally active, and specific angiotensin II antagonist acting on the AT1 receptor subtype. It is categorized in angiotensin receptor blocker. Valsartan is poorly soluble and the aqueous solubility is reported to be less than 1 mg/mL. The drug has oral bioavailability of about 23% due to its poor aqueous solubility.[3]

Micro emulsions are clear, transparent, optically isotropic and thermodynamically stable systems comprising of oil, surfactant, co-surfactant and aqueous phase.[4] Micro emulsion (ME), a novel drug delivery system, has been reported to improve the rate and extent of absorption of lipophilic drugs.[11] Micro emulsions are used as edge as potential drug delivery vehicles because of their thermodynamic stability, reversibility, simple manufacturing, and scale up feasibility, and do not require any special equipment. Oil-in-water (o/w) micro emulsion is the most suitable formulation, which is expected to increase the solubility by dissolving the compounds with low water solubility into an oil phase.[5] Thus, Valsartan is considered to be a good candidate for micro emulsion drug delivery system to enhance its oral bioavailability by reducing the droplet size, hence increasing the rate of absorption due to surfactant induced permeability changes.

MATERIALS AND METHODS

Materials

Valsartan was a gift sample from Zim Laboratories Limited (Kalmeshwar, Maharashtra, India). Cremophor EL was a gift sample from BASF India Ltd (Mumbai, India), Capmul MCM was a gift sample from Indchem International (Mumbai, India) and Transcutol HP was a gift sample from Gattefosse India Pvt. Ltd. All other chemicals used were of analytical reagent grade and used as received without further purification. Double-distilled water was used throughout the study. The animal requirement was approved by the Institute Animal Ethics Committee, and all experiments were conducted as per the norms of the Committee for the Purpose of Supervision of Experiments on Animals, India.

Methodology

Solubility of the oil phase, surfactant and co-surfactant

Selection of the oil phase was based upon the maximum solubility of the drug. Different oils including Capmul

MCM, castor oil, rice bran oil, oleic acid, labrafil 1944 CS and captex 355 were taken for the studies. Also various surfactants like tween 80, span 20, cremophor EL, and cremophor RH 40 were taken and co-surfactants like iso-propanlol, ethanol, transcutol HP, transcutol P, capryol 90, polyethylene glycol (PEG) 400 and propylene glycol (PG) were taken for solubility studies. 2 ml of each of the selected vehicle were added to each cap vial containing an excess of valsartan. After sealing, mixtures were shaken with shaker at 25°C for 48 hr. After reaching equilibrium, each vial was centrifuged at 500 rpm for 15 min, and excess insoluble valsartan was discarded by filtration using a membrane filter (0.45 µm, 13 mm, Whatman). The concentration of valsartan was determined in each oils, surfactants, and co-surfactants by UV spectrophotometer at their respective wavelength 250 nm.[6]

Pseudo-ternary phase diagram

Pseudo-ternary phase diagrams were constructed to obtain the appropriate components, and their concentration ranges that resulted in a large existence area of micro emulsion were chosen. In order to optimize the concentration of oil phase, surfactant and co-surfactant, different batches of varied concentration were prepared and titrated with distilled water till transparency persisted. Ternary phase diagram was prepared by using a constant ratio of surfactant to co-surfactant. Four ratios of surfactant (Cremophor EL) and co-surfactant (Transcutol HP) were selected (1:1, 2:1, 3:1 and 4:1). At each specific Smix weight ratio, the ratio of oil to the Smix was varied as 1:9, 2:8, 3:7, 4:6, 5:5, 6:4, 7:3, 8:2 and 9:1. Each mixture of oil, surfactant and co-surfactant, at specific weight ratio were titrated with water along water dilution lines. If clear and transparent mixtures were visualized after stirring, the samples were considered to be monophasic and were marked as points in the phase diagrams and areas covered by these points were considered as the micro emulsion region of existence region. Maximum micro emulsion region was optimized for further studies and four ratios of the optimized formulation were characterized.

Preparation of Valsartan micro emulsion system

Valsartan containing micro emulsion was prepared by water titration method. Valsartan was first dissolved in the pre-measured volume of oil by stirring on a magnetic stirrer. A mixture of the surfactant and co-surfactant at a fixed ratio (v/v) was added to the above resulting mixture. Finally this mixture was titrated with distilled water. The optimized micro emulsion drug delivery system was also subjected for accelerated stability study.[8]

Characterization of micro emulsion

Percentage Transmittance

Transparency of micro emulsion formulation was determined by measuring percentage transmittance through U.V. Spectrophotometer (UV-1601 SHIMADZU) at 650 nm with distilled water taken as blank and three replicates were performed for each sample.[6,8,11]

pH determination

The apparent pH of all the selected micro emulsions and the plain micro emulsion was determined at 25°C by immersing the electrode directly into the micro emulsion using a digital pH meter.[8,12]

Refractive index

Refractive indices of the prepared micro emulsions were determined at 25°C by Abbe's refractometer by placing one drop of micro emulsion on the slide.[6,9]

Viscosity measurement

Micro emulsions are generally low viscosity systems. The viscosity of the prepared micro emulsion was measured at 25°C at 60 rpm by LV spindle no. 63 using a Brookfield viscometer (model LVDVE230; Brookfield Engineering Laboratories, Inc).[6,8,12]

Determination of Drug Content in the micro emulsion

The drug content of the micro emulsion formulation was determined by dissolving 1 ml (equivalent to 10 mg drug) of the formulation in 10 ml of methanol. After suitable dilutions with methanol, absorbance was determined using the UV spectrophotometer keeping blank micro emulsion as control at wavelength 250 nm and three replicates were performed for each sample.[5,6,8]

Particle size Determination

The particle size of the plain micro emulsion and final formulation F4 was determined by using particle size analyzer Nanophox (NX0088) Sympatec, Germany performed at Sinhagad Institute of Pharmacy, Pune.[9]

Drug release studies

***In-vitro* drug release**

The diffusion study was carried out using a modified Franz diffusion cell. The receptor compartment was filled with 30 ml of Phosphate buffer (pH 5.5). The donor compartment was fixed with cellophane membrane and it contained micro emulsion (Valsartan) and plain drug suspension separately. At predetermined time intervals of 30 minutes samples were withdrawn from receptor compartment and analyzed for drug content by UV Spectrophotometer at 250 nm.[10]

***In-vitro* intestinal permeability study**

The methods employed were modified from experimental procedures. Male albino rats (250-300 g) were killed by overdose with pentobarbitone administered by intravenous injection. To check the intra-duodenal permeability, the duodenal part of the small intestine was isolated and taken for the in vitro diffusion study. Then this tissue was thoroughly washed with cold Ringer's solution to remove the mucous and lumen contents. The equivalent dose of optimized micro emulsions and plain drug solution were prepared. One side of the intestine

was tightly closed, resultant samples (1 mg/mL) were injected into the lumen of the duodenum using a syringe and then other side of the intestine was tightly closed. Then the tissue was placed in a chamber of organ bath with continuous aeration and a constant temperature of 37˚C. The receiver compartment was filled with 30 ml of phosphate-buffered saline (pH 5.5). After a particular time interval of 30 minutes the 1ml sample was withdrawn and diluted to 10 ml. The absorbance was measured using a UV-VIS spectrophotometer at a wavelength of 250 nm, keeping the respective blank. The percent of cumulative drug diffusion was calculated against time and plotted on a graph. Similarly, suspension of plain drug was prepared and compared with the optimized formulation.[10]

Drug stability

Stability studies of selected formulation were carried out at ambient temperature (30°C±2°C), under cold condition 4 ± 2°C and at elevated temperature 40 ± 5°C. After every 15 days the micro emulsion was analyzed for phase separation, % transmittance, pH and refractive index.[5]

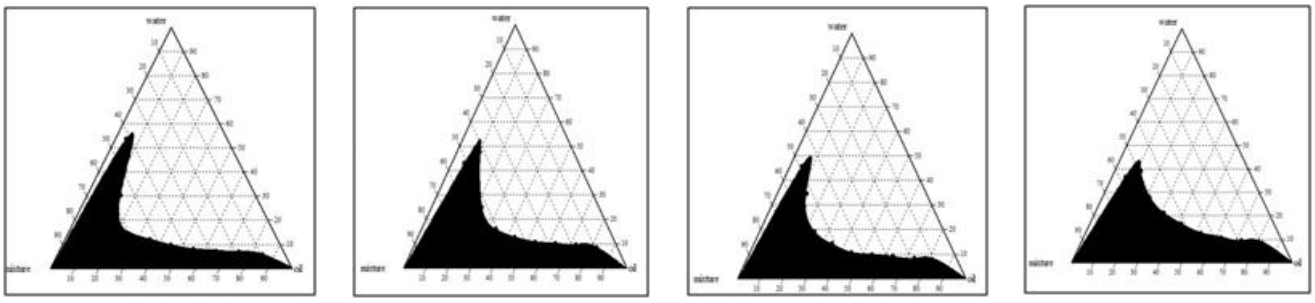
RESULTS AND DISCUSSION

Solubility of the oil phase, surfactant and co-surfactant:

Solubility studies were carried out to identify the potential ingredients for the formulation of micro emulsion. The solubility studies were performed using different oils, surfactants and co-surfactants to find out the highest solubility of the drug valsartan in all the components of micro emulsion. The results are shown in table 1. The drug shows highest solubility in the oil Capmul MCM, surfactant Cremophor EL and co-surfactant Transcutol HP.

Table 1: Solubility of valsartan in various oils, surfactants and co-surfactants

Oils	Solubility mg / ml	Surfactant	Solubility mg/ml	Co-surfactant	Solubility mg / ml
Castor oil	534	Tween 80	501	Propanolol	1006
Capmul MCM	823	Cremophor EL	615	Ethanol	1025
Captex 355	3	Span 20	166	PEG 400	909
Rice bran oil	4	Cremophor RH 40	489	Propylene glycol	339
Oleic acid	23			Transcutol P	1261
Labrafil 1944	19			Transcutol HP	1621
				Capryol 90	1021



F1 F2 F3 F4

Figure 1: Ternary phase diagrams representing the micro emulsion formed with Capmul MCM (oil), Cremophor EL (surfactant) and Transcutol HP (co-surfactant).

Pseudo-ternary phase diagram

Pseudo-ternary phase diagrams were constructed by sing Capmul MCM (oil), Cremophor EL (surfactant) and Transcutol HP (co-surfactant) to identify the micro emulsion existing zone from which appropriate concentration ranges of components of micro emulsion can be obtained. The ternary phase diagrams of all the ratios are shown in figure 1. Formation of micro emulsion systems (the shaded area) was observed at room temperature. Phase behavior investigations of this system demonstrated the suitable approach to determine the water phase, oil phase, surfactant concentration, and cosurfactant concentration with which the transparent, one-phase, low-viscous microemulsion system was formed. Thus, four ratios of the optimized formulation were selected.

Characterization of the micro emulsion

The optimized formulations were further studied for percent transmittance, drug content, pH determination, refractive index and viscosity of the different ratios of the selected system:

Percentage Transmittance

The percent transmission carried out on UV spectrophotometer at 650 nm was found to be in the range of 98.23 % to 99.37 % for formulations F1 to F4 along with the plain formulation which confirms good transparent nature of formulations.

Drug Content

The drug content at 250 nm was found to be in the range of 99.23% to 99.72% in the optimized F1 to F4 formulations.

pH determination

For the formulations F1 to F4, the pH value was found to be in the range of 3.66 to 4.02.

Refractive index

The refractive index carried out by Abbe refractometer was found to be in the range of 1.3618 to 1.3646 of F1 to F4 formulations along with the plain formulation.

Viscosity

All formulations F1 to F4 were found to have rather low viscosities, ranging from 63 to 96 cps. The viscosity of the micro emulsion increased with increasing concentration of the surfactant.

Particle size determination

The particle size was found to be 51.32 nm for the drug formulation F1, which was found to be in the range for micro emulsions (10-100nm). The result is shown in figure 2.

Table 2: Results for percent transmittance, drug content, pH determination, refractive index and viscosity of optimized formulation along with plain system

Formulation code	Percent transmittance (%)	pH	Refractive index	Viscosity (cp)	Drug content (%)
Without drug	99.2 ± 0.04	3.66 ± 0.07	1.3648±0.0006	63.33±4.1	-
F1 (1:1 ratio)	99.37 ± 0.16	3.96 ± 0.20	1.3620 ± 0.004	66.36±5.7	99.72 ± 0.31
F2 (2:1 ratio)	99.32 ± 1.33	3.92 ± 0.05	1.3618 ± 0.002	76.46±4.77	99.4 ± 1.75
F3 (3:1 ratio)	98.47 ± 1.02	3.94 ± 0.02	1.3620 ± 0.005	83.33±4.34	99.32 ± 1.33
F4 (4:1 ratio)	98.23 ± 1.91	4.02 ± 0.12	1.3618±0.0012	96.66±5.74	98.23 ± 1.91

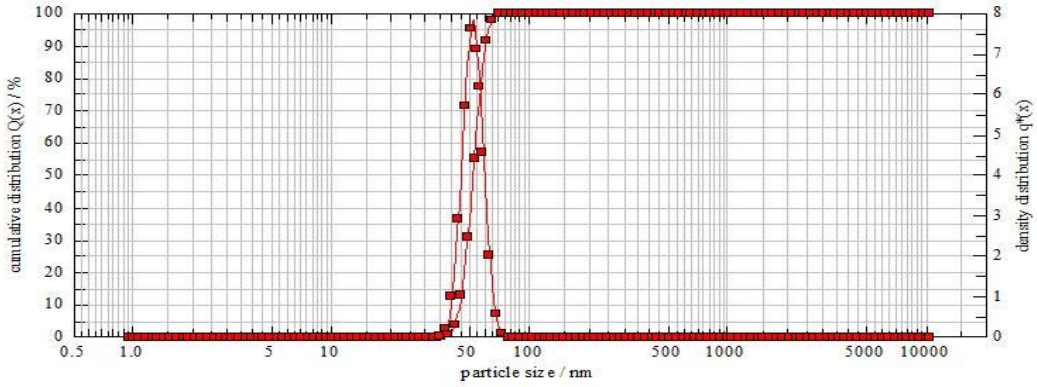


Figure 2: Shows the particle size determination of the drug loaded formulation.

Drug release studies

In-vitro drug release study

It was seen that after 4 hours of diffusion, the drug released from the formulation F1 faster and more than that of the other ratios i.e., 93.410%. The results are shown in figure 3.

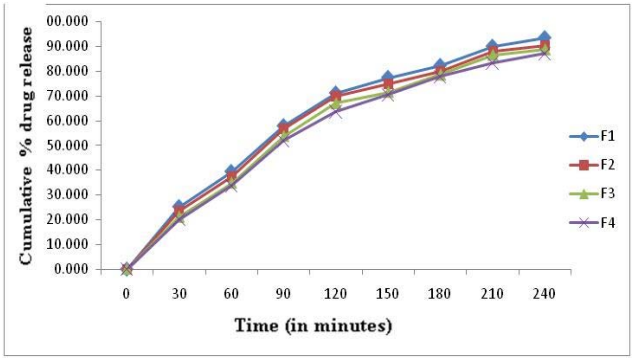


Figure 3: *In-vitro* cumulative average drug release formulations F1, F2, F3 and F4

In-vitro intestinal permeability study

From the figure 4, it was seen that after 4 hours of diffusion, 99.478% of the drug was released from the formulation F1 which is higher than that of the other ratios. Hence, F1 formulation was selected as optimized formulation and was considered for comparison with the plain drug suspension. From the comparison study of F1 formulation and plain drug suspension, it can be concluded that the extent of diffusion of valsartan from the Capmul micro emulsion is greater than the plain drug suspension as shown in figure 5.

Stability studies

Stability studies of selected formulation were carried out at 30°C±2°C, 4°C±2°C and 40°C±5°C. The results of stability are shown in table 3.

Table 3: Stability studies of micro emulsion formulation F1

Temperature	Days	Phase separation	Viscosity	Drug content	pH	Percent transmittance	Refractive index
4 ± 2°C	15	No	60.66 ± 4.12	99.63± 0.04	3.87± 0.04	99.22± 0.07	1.3618 ± 0.0002
	30	No	55.36 ± 4.70	99.42± 0.03	3.83± 0.03	99.14± 0.03	1.3601 ± 0.0012
	45	No	51.06 ± 5.7	99.22 ± 0.04	3.78± 0.06	98.97± 0.03	1.3585 ± 0.0008
30°C±2°C	15	No	56.36 ± 4.3	99.46± 0.03	3.78± 0.02	99.17± 0.04	1.3615 ± 0.0006
	30	No	47.66 ± 5.71	99.16 ± 0.03	3.73± 0.08	99.02± 0.03	1.3510 ± 0.0006
	45	No	43.36 ± 4.23	98.82± 0.04	3.66± 0.05	98.92± 0.03	1.3512 ± 0.0007
40 ± 5°C	15	No	44 ± 3.464	99.24± 0.03	3.72± 0.04	99.06± 0.04	1.3550 ± 0.0037
	30	No	39.26 ± 4.20	98.84± 0.05	3.68± 0.04	98.91± 0.05	1.3548 ± 0.0055
	45	No	35 ± 7.071	98.52± 0.04	3.63± 0.03	98.72± 0.08	1.3482 ± 0.0032

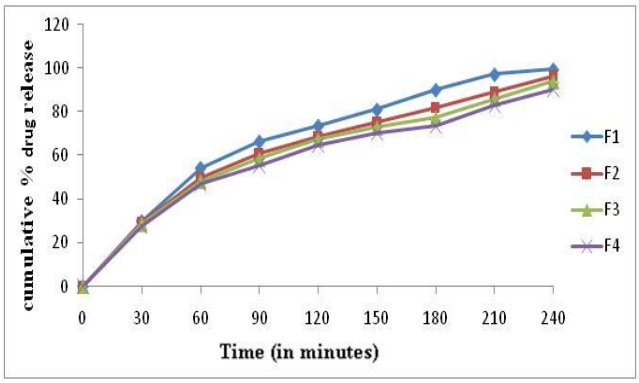


Figure 4: *In-vitro* intestinal cumulative average drug release formulations F1, F2, F3 and F4

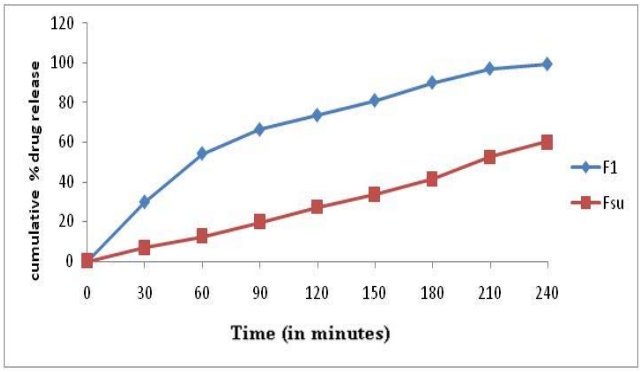


Figure 5: Comparison of in-vitro intestinal drug release for micro emulsion formulation F1 and plain drug suspension, Fsu

CONCLUSION

The optimized micro emulsion formulation F1 containing Capmul MCM as oil (2%), Cremophor EL as surfactant (18%), Transcutol HP as co-surfactant (18%) and distilled water (62%) was a transparent, clear and low viscosity system, with particle size 51.32 nm. The percent transmission and the refractive index was found to be 99.37% ± 0.16% and 1.3620 respectively which shows the good clarity of the prepared micro emulsion. The micro emulsion showed the viscosity 66.66 ± 5.7 cp. The pH was found to be 3.76 ± 4.20 and the drug content was found to be 99.42% ± 0.31%. After the *in vitro* intestinal permeability studies, the prepared microemulsion showed the *in vitro* intestinal permeability release of about 99.47% within 4 hours which is more than that of plain drug suspension 60.33%. The stability studies confirmed that the optimized formulation is stable for a period of 45 days. Thus, it can be concluded that valsartan having poor solubility can be formulated as microemulsion, which increase the solubility of the drug and hence, which will increase its oral bioavailability than the other dosage forms.

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